DFG FOR 1363

Biogenic Amines, and the control of insect behaviour.

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Moscow State University, March 2014

BIOGENIC AMINES: From Wikipedia (http://en.wikipedia.org/wiki/Biogenic_amine)

Histamine – (derived from the amino acid histidine), acts as a **neurotransmitter** mediating **arousal and attention**, *as well as a **pro-inflammatory signal** released from mast cells in response to allergic reactions or tissue damage. Histamine is also an important **stimulant of HCl secretion** by the stomach through histamine H_2 receptors.*

Serotonin – (derived from the amino acid tryptophan), a central nervous system **neurotransmitter** involved in regulating **mood, sleep, appetite**, and **sexuality**.

The three **catecholamine** neurotransmitters (*derived from amino acid tyrosine*):

Norepinephrine (noradrenaline) - a **neurotransmitter** involved in **sleep** and **wakefulness**, **attention**, and **feeding behavior**, as well as a **stress hormone** released by the adrenal glands that **regulates the sympathetic nervous system**.

Epinephrine (adrenaline) - an adrenal **stress hormone**, as well as a **neurotransmitter** present at lower levels in the brain.

Dopamine - a **neurotransmitter** involved in **motivation, reward, addiction, behavioral reinforcement**, and **coordination of bodily movement**.

The **trace amines** (*with respect to their "rare abundance" in vertebrates*):

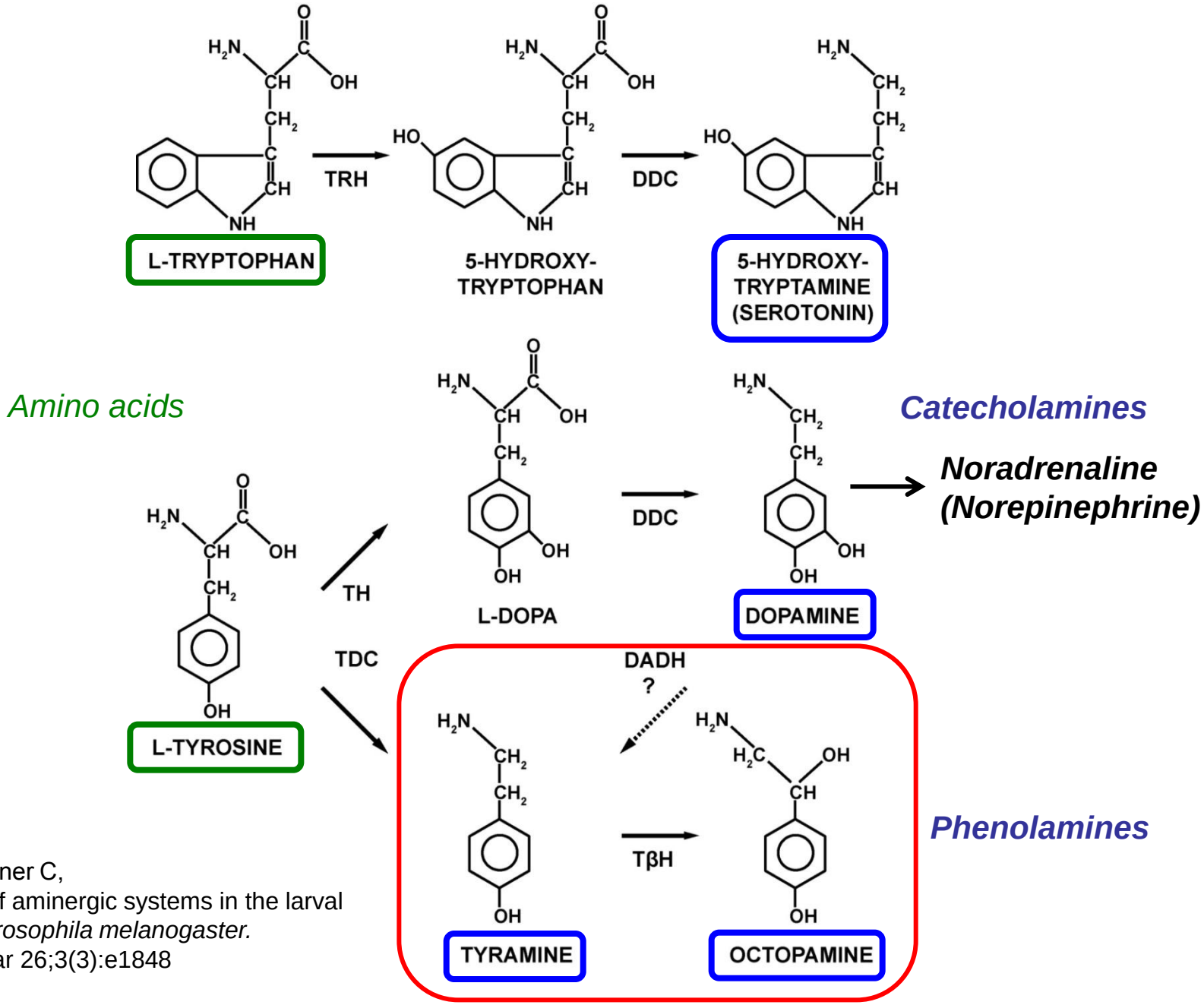
3-Iodothyronamine – (a metabolite of the thyroid hormones), has been hypothesized to be the primary endogenous ligand for the trace amine-associated receptor 1 (TAAR1).

Tryptamine – (a monoamine alkaloid), found in trace amounts in the brains of mammals, and believed to **play a role as a neuromodulator or neurotransmitter**.

Tyramine - a phenolamine substance that is found in many common foods, and is associated **with increased blood pressure and headaches**.

As well as others such as **dimethyltryptamine (DMT)**, **phenethylamine**, and **octopamine** and the *meta*-substituted positional isomers of octopamine and tyramine

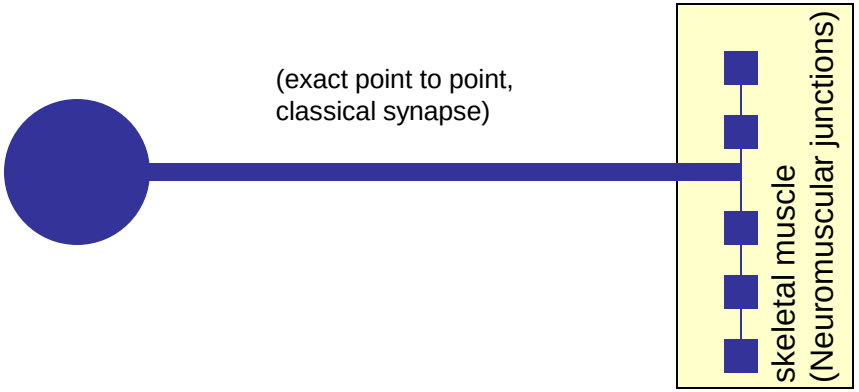
Major Biogenic Amines and their synthesis



From:
Vömel M and Wegener C,
Neuroarchitecture of aminergic systems in the larval ventral ganglia of *Drosophila melanogaster.*
PLoS One. 2008 Mar 26;3(3):e1848

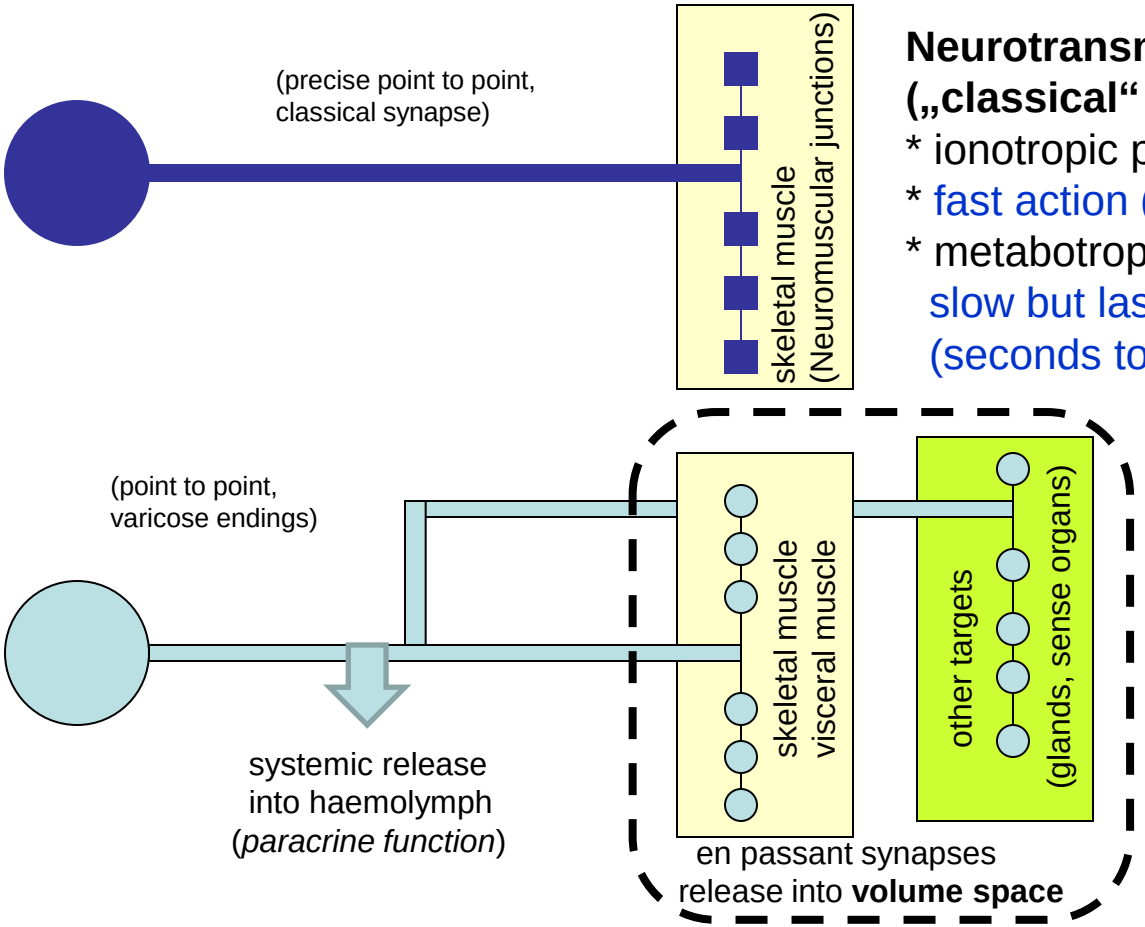
One of the most copied sentences from previous reviews, and I am no exception:

„…..octopamine is released as a neurohormone, a neuromodulator and a neurotransmitter“.



Neurotransmitter
(„classical“ neurotransmitter)

* ionotropic postsynaptic receptor
* fast action (milliseconds)
* metabotropic postsynaptic receptor
  slow but lasting action
  (seconds to minutes to hours)



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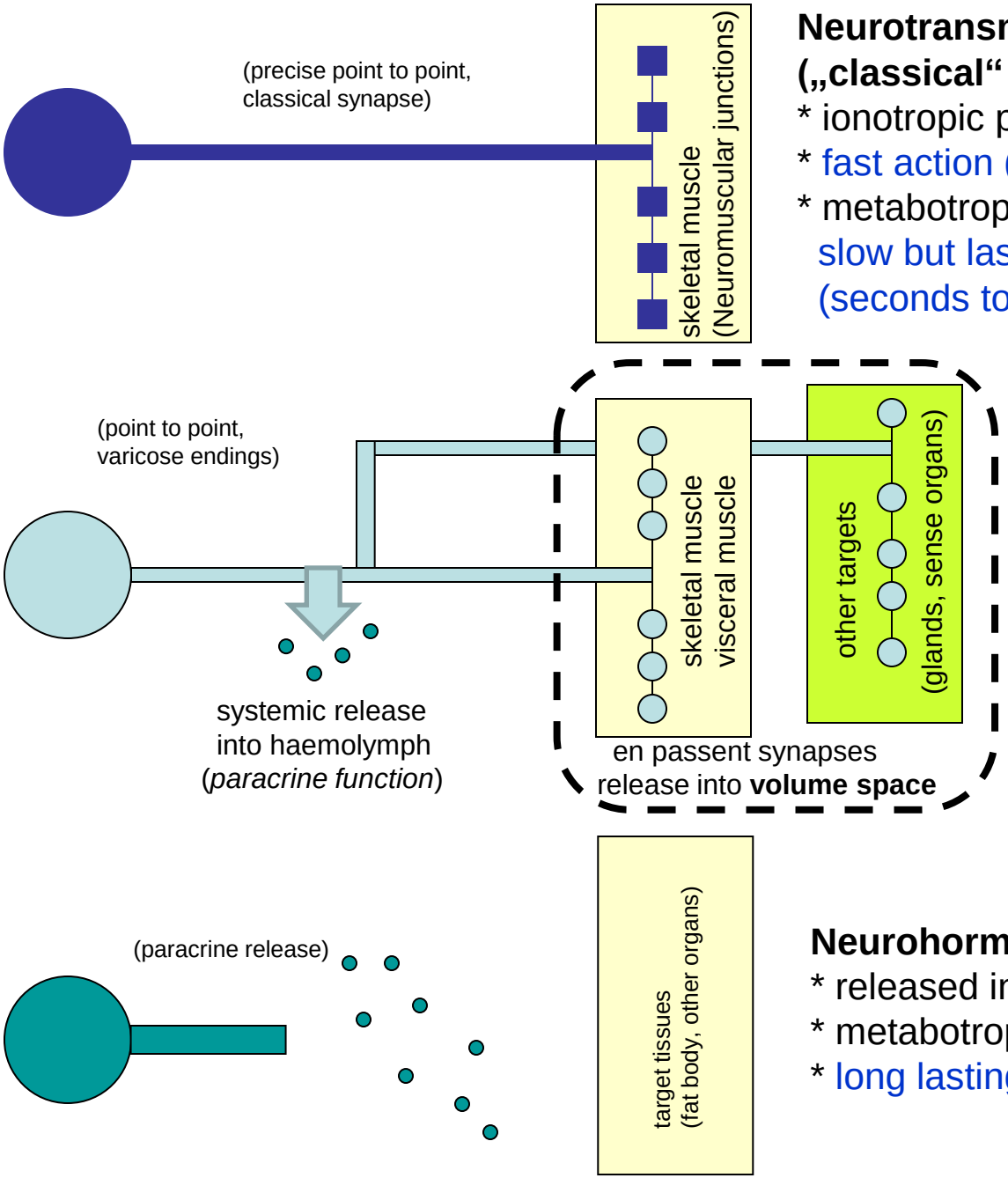
Neuromodulator
* metabotropic postsynaptic receptor
* slow but lasting action
* (minutes to hours to days)
* targeted release, plus release into haemolymph space possible



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* (minutes to hours to days)
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Neurohormone
* released into haemolymph, systemic release
* metabotropic postsynaptic receptor
* long lasting action (hours to days to weeks)

What are our aims ?

What are our aims ?

* To describe the behavioural actions of biogenic amines such as **tyramine** and **octopamine** at a behavioral and cellular level:

- identify the neurons (immunocytochemistry and recording with sharp electrodes)

- characterize their patterns of activity (firing) and their synaptic inputs (for example, sensory inputs)

- characterize the „network“

- make comparisons by studying different (closely related and more distant) animal species

- define possible evolutionary „building blocks“ of neuronal networks and behavior

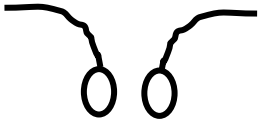
Our experimental animals:

* hemi- and holometabolous insects

The neurons which release **tyramine** or **octopamine** (tyramine?) in insects (locust, *Schistocerca gregaria*)

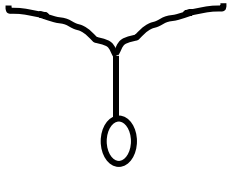
Neurons within the **CNS** (brain and ventral cord) *(„Interneurons“)*

- **Paired neurons in brain**
  *- ~ 30 **OA** cells except optic ganglia (at least 100),*
  *- ~ 80 **TA** cells*

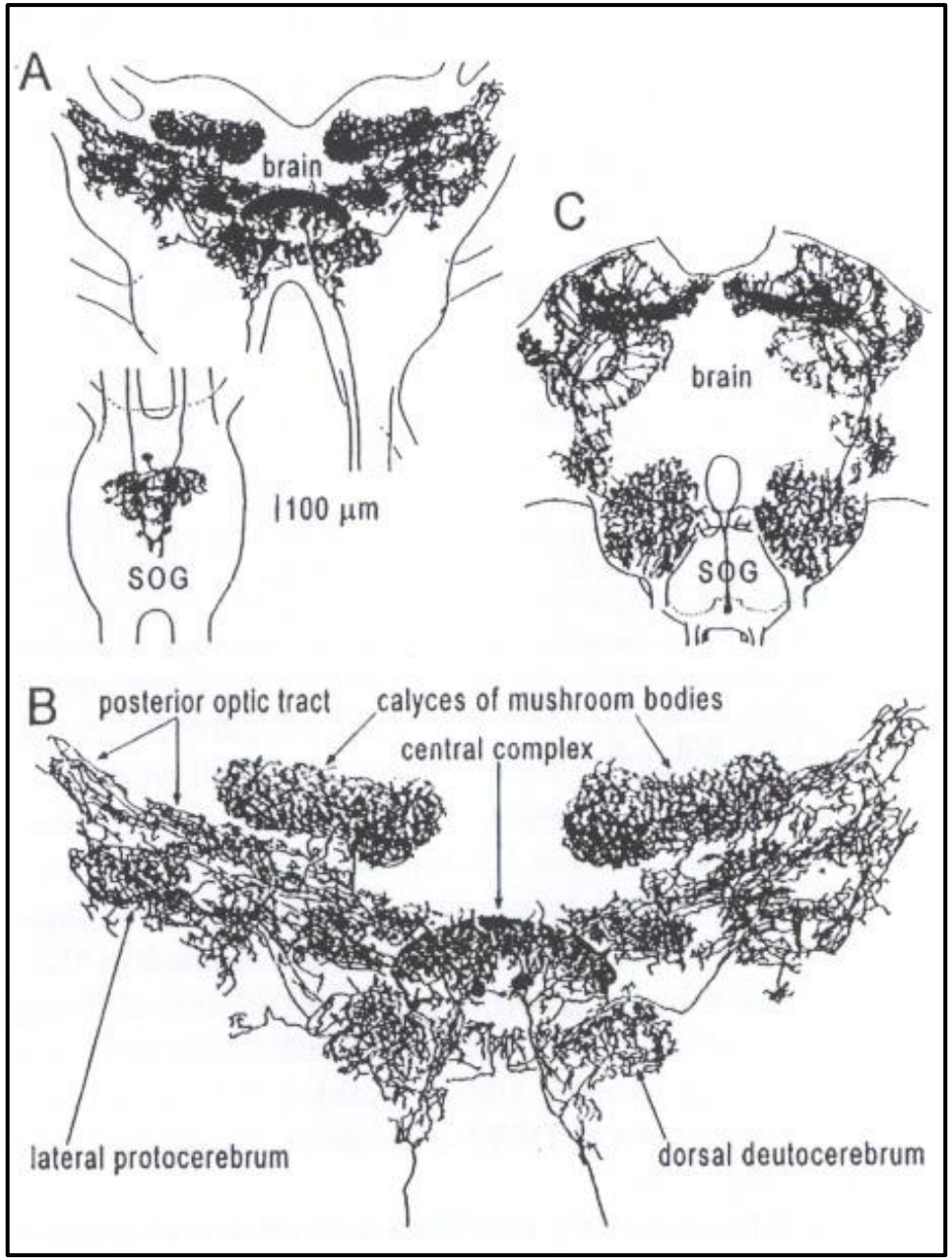


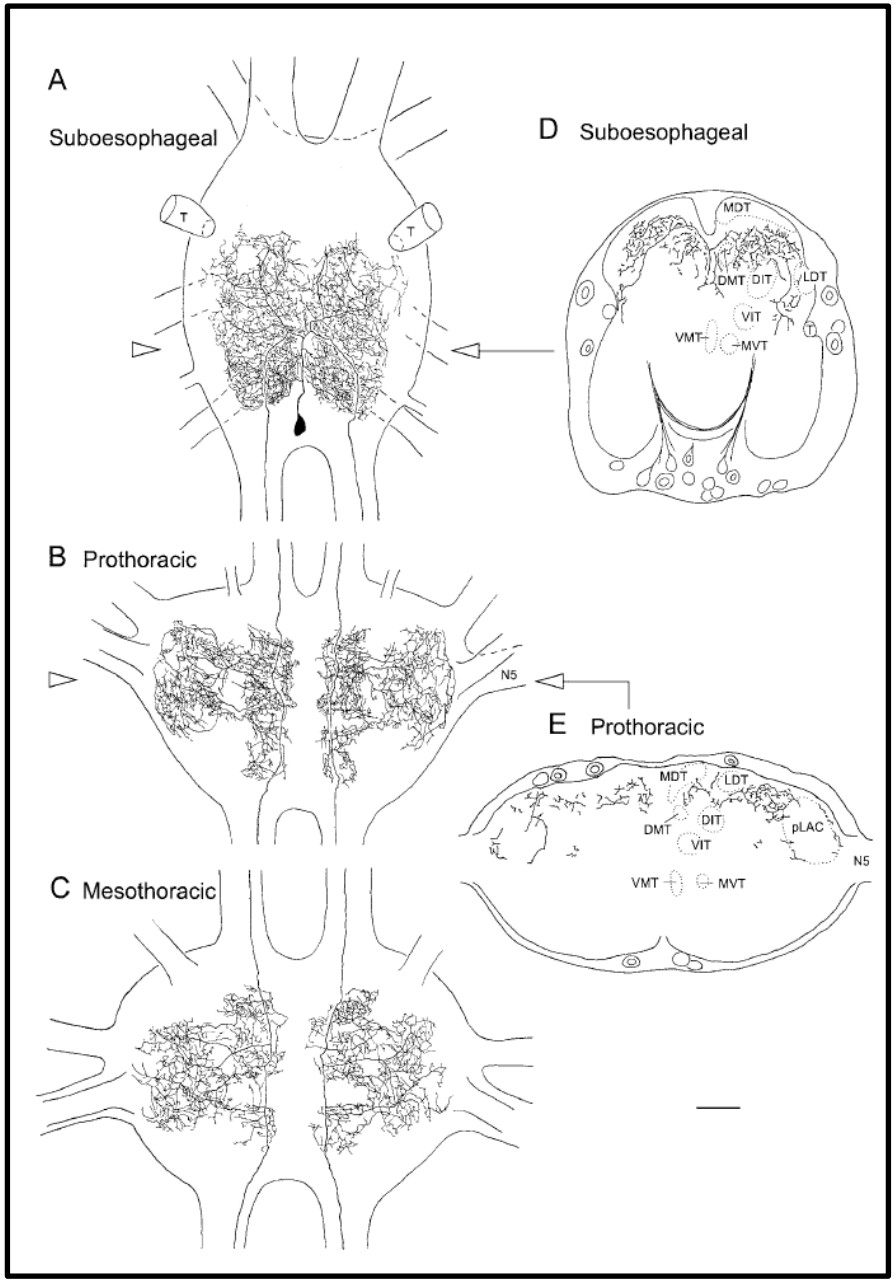
- **Paired neurons in ventral cord**
  *- ~ 10 paired ventral lateral intersegmental cells (**OA**),)*
  *- ~ 10 paired intersegmental ventral cells in all fused ganglia (**TA**),*

- **Unpaired neurons in suboesophageal ganglion** (*all **OA***)
  - 9 ascending to brain innervating all major neuropiles
  - 6 descending to ventral cord innervating thoracic and abdominal neuropiles (6)



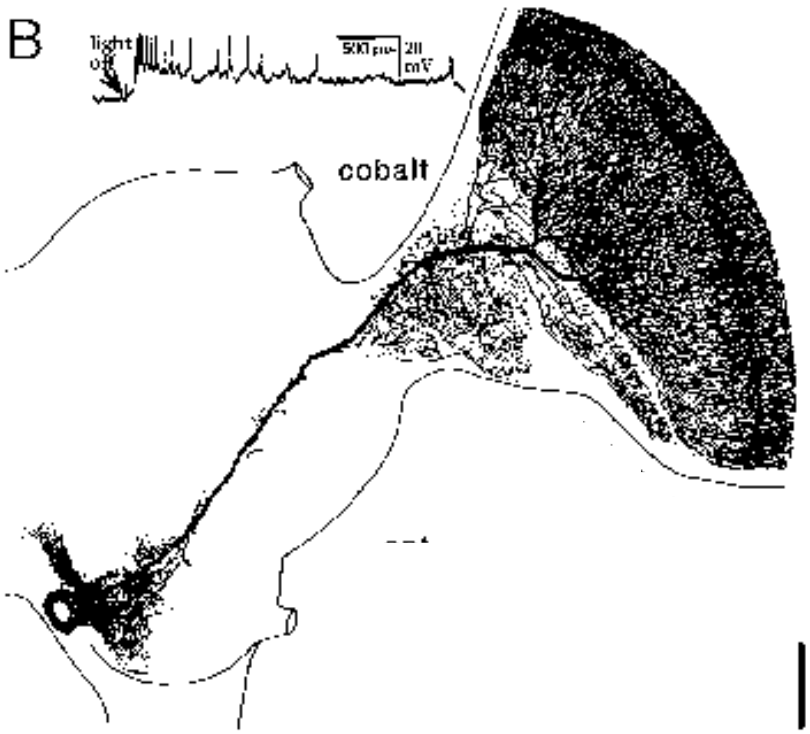
ASCENDING unpaired median neurons of SOG DESCENDING

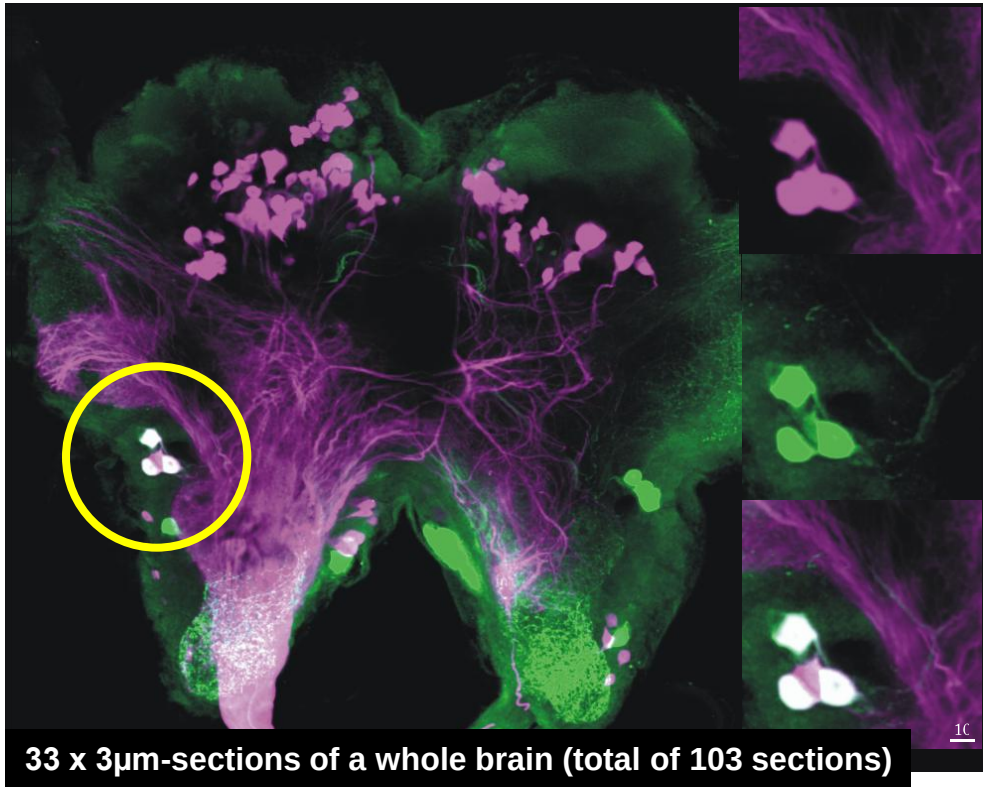




(Bräunig, P. (1991). *Philos. Trans. R. Soc. Lond. [B]* **322**, 221-240)

Bräunig and Burrows, J Comp Neurol 478:164-175, 2004





Stevenson and Spörhase-Eichmann,
Comp Biochem Physiol 110A, 203-215, 1995

Bacon et al., J Neurophysiol 74, 2739-2743, 1995

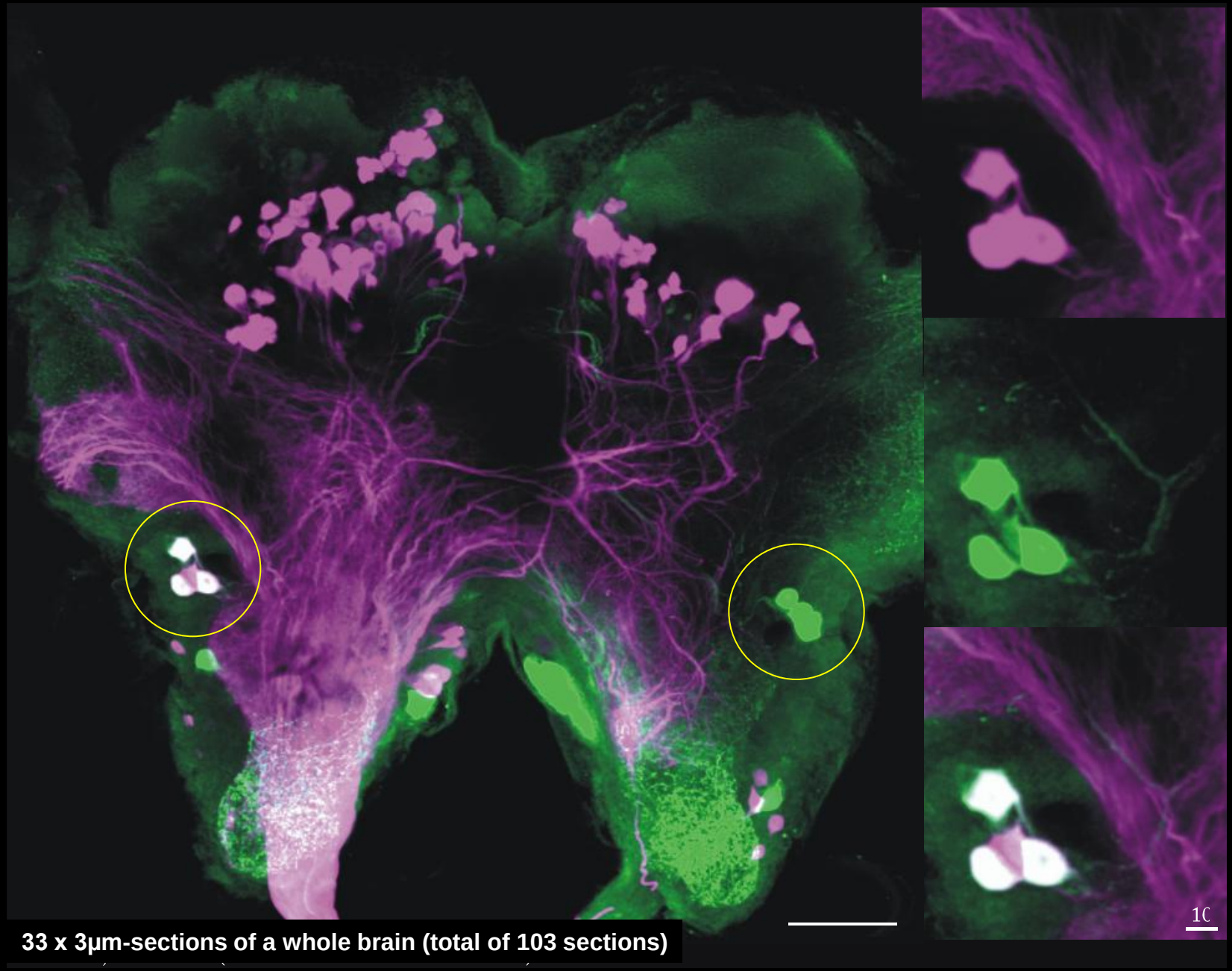
Kononenko, Wolfenberg and Pflüger, unpublished results, 2009
S. Hartfil, PhD-thesis

* involved in modulating optical responses

Longden, Krapp; Front Syst Neuroscii 2010
Jung, Borst, Haag; J Neurosci 2011
Rien, Kern, Kurtz; Front Behav Neurosci 2013

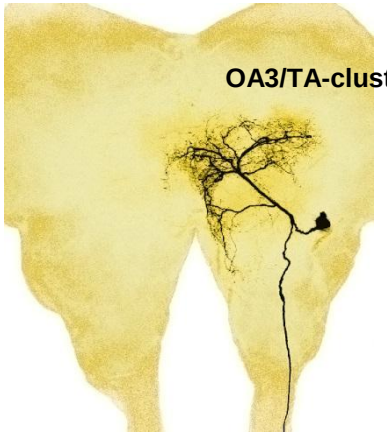
* these neurons produce OA only after „stress stimuli“

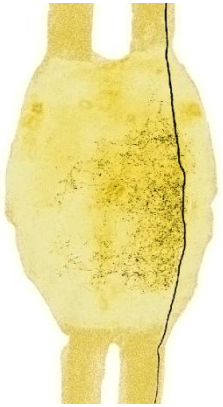
Kononenko et al., J Comp Neurol, 2009

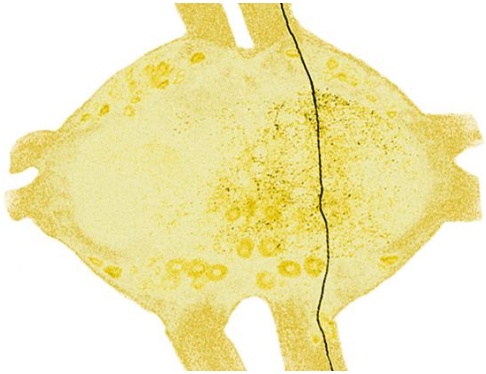


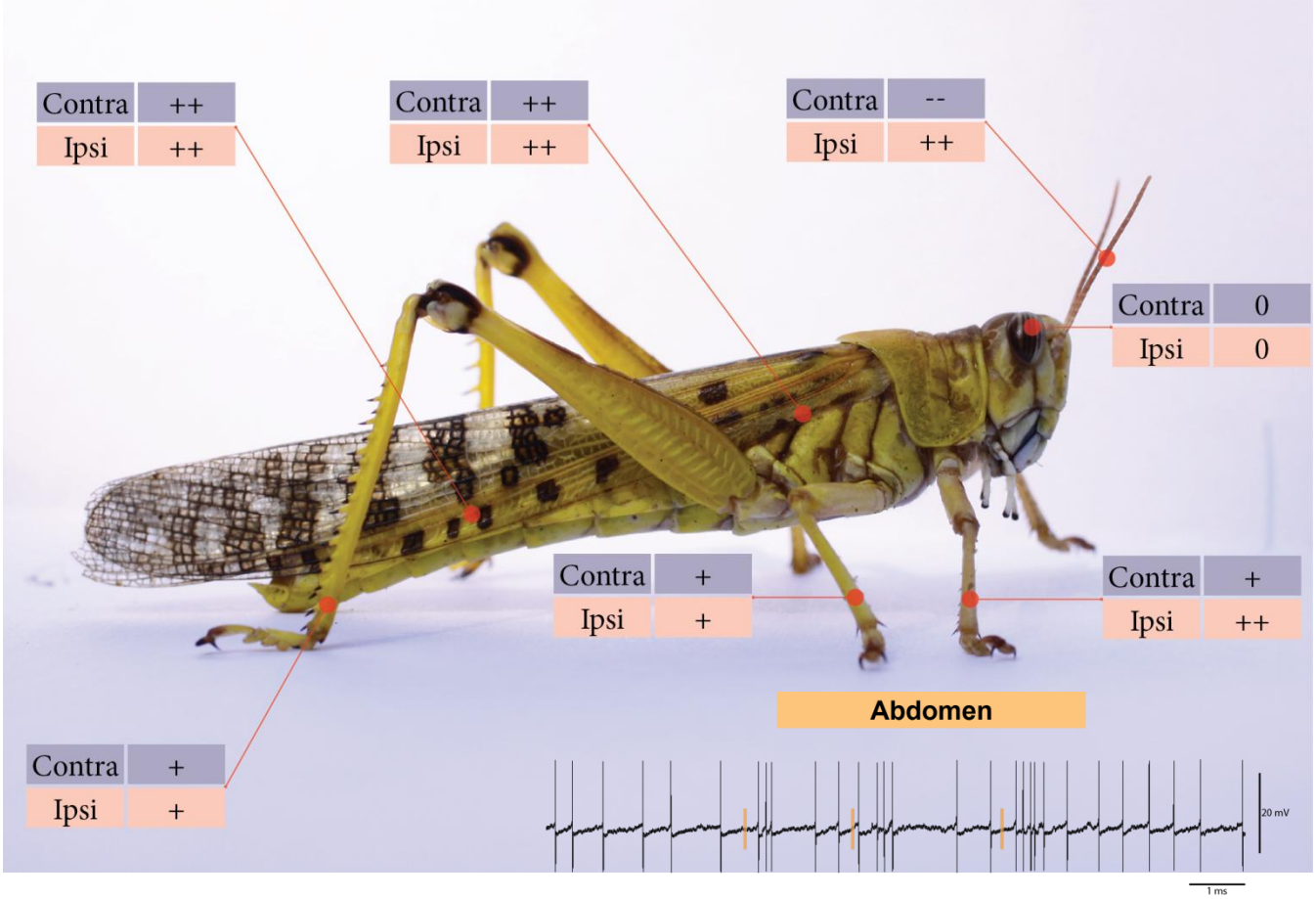
Neurobiotin backfill (magenta) from pro-meso-connective and anti-tyramine (green)

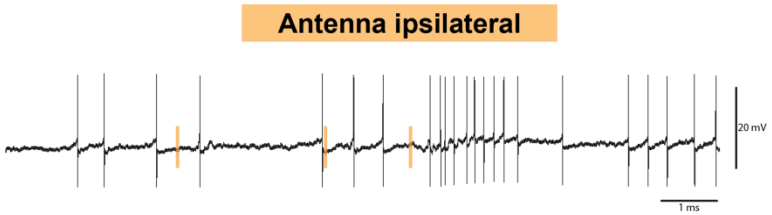
Kononenko, Wolfenberg and Pflüger, unpublished results, 2009

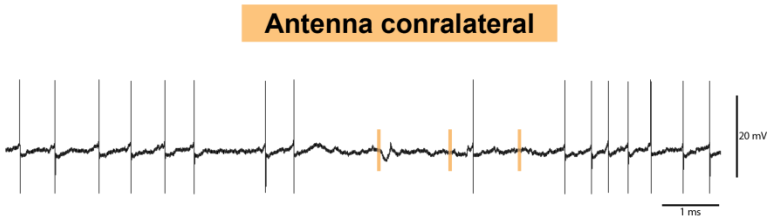












PhD-thesis Sergej Hartfil

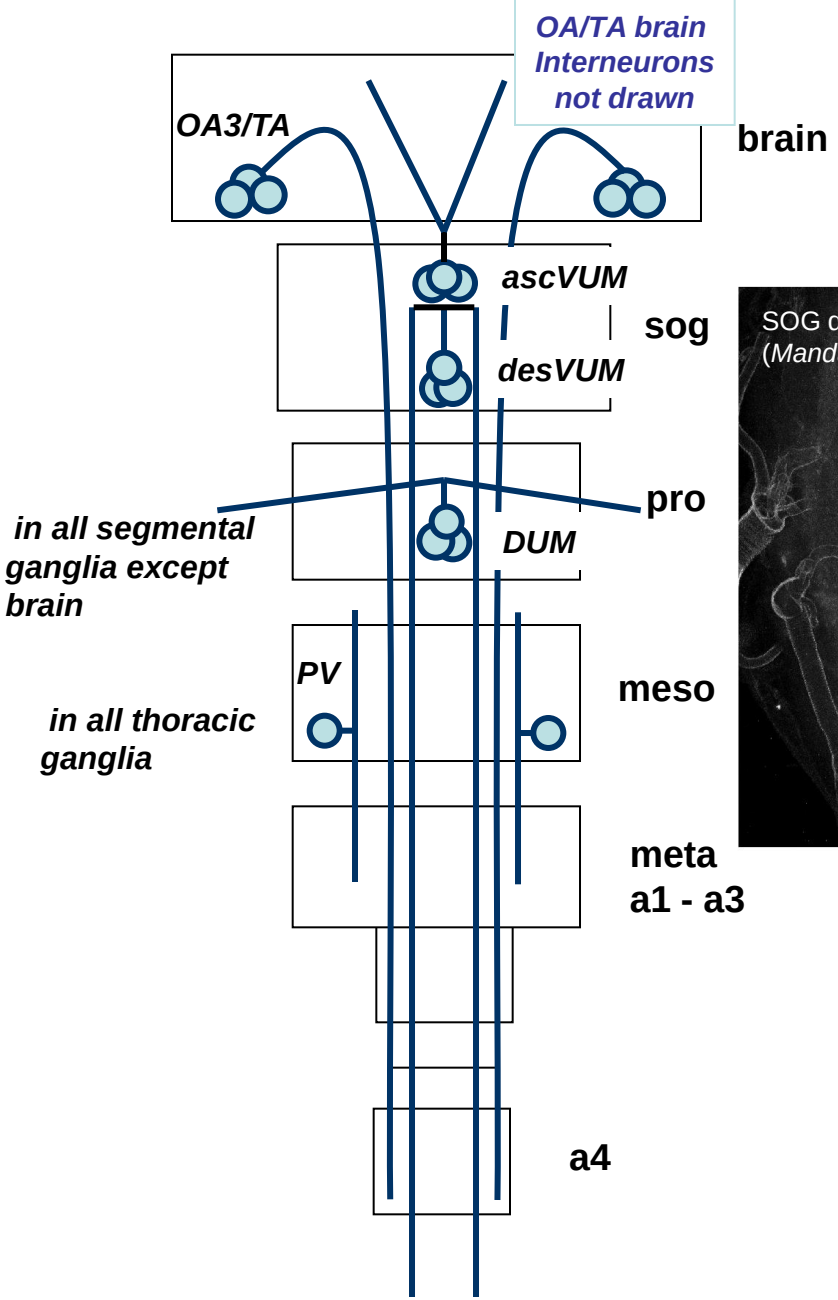
* These tyraminergic/octopaminergic projection neurons descending from the brain are very interesting:

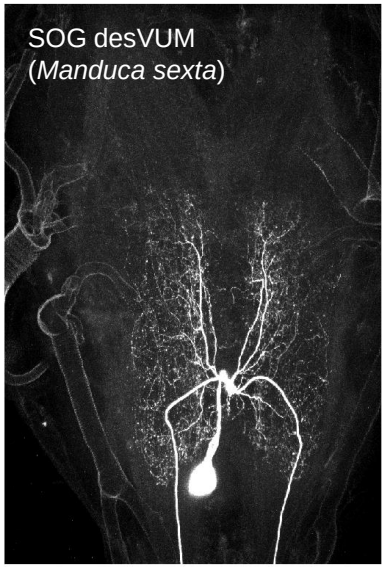
- produce octopamine only task-specific (after stressing stimuli) (*in cockroaches tßh-enzyme which is required to synthesize octopamine, is upregulated by stress, Chatel et al., Journal of Molecular Endocrinology (2013) 50, 91–102*)

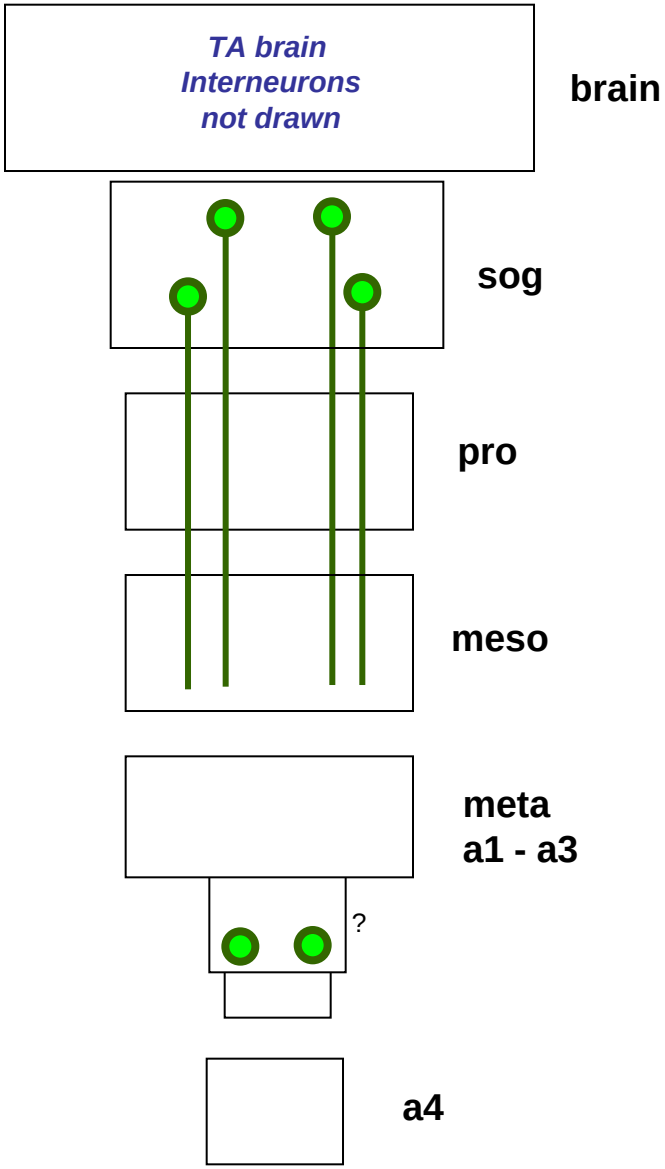
- collect mechanosensory information from the whole body *(perhaps, involved in arousal)*

- target all relevant thoracic neuropiles

* PhD-thesis Sergej Hartfil

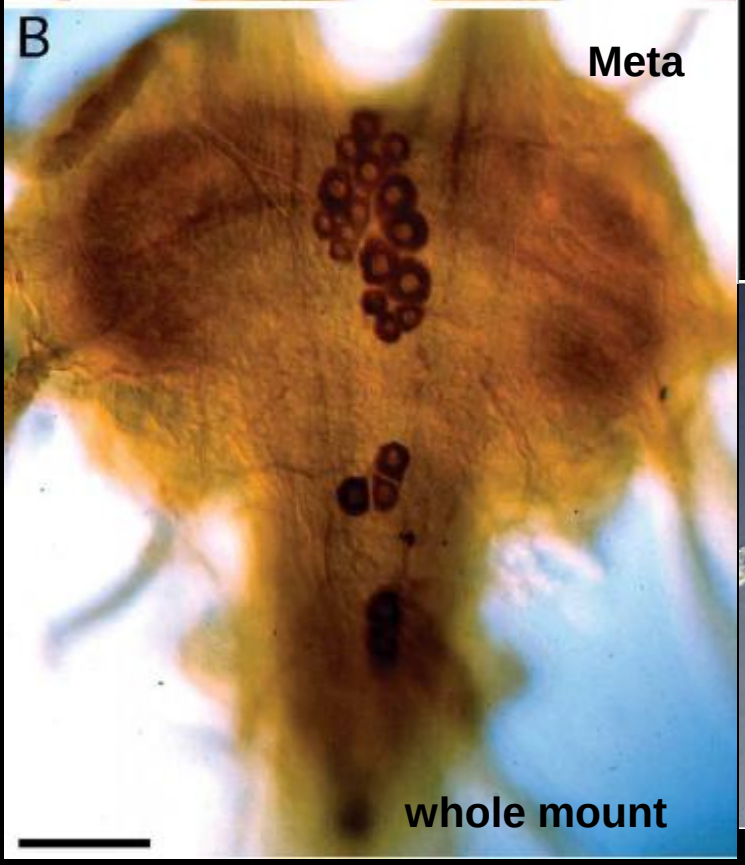


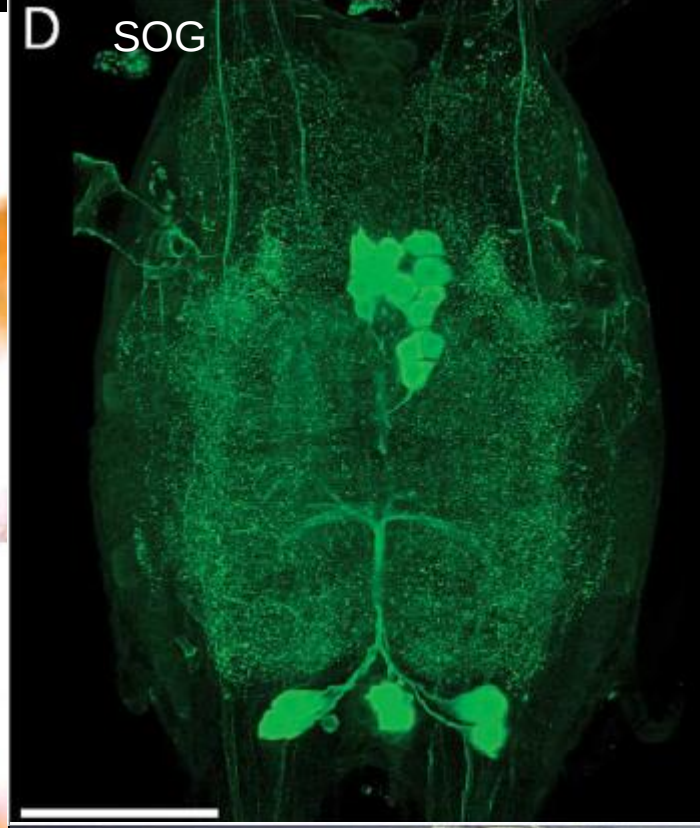




The system of octopaminergic neurons within the locust ventral cord.

The system of „purely“ tyraminergic neurons within the locust ventral cord.

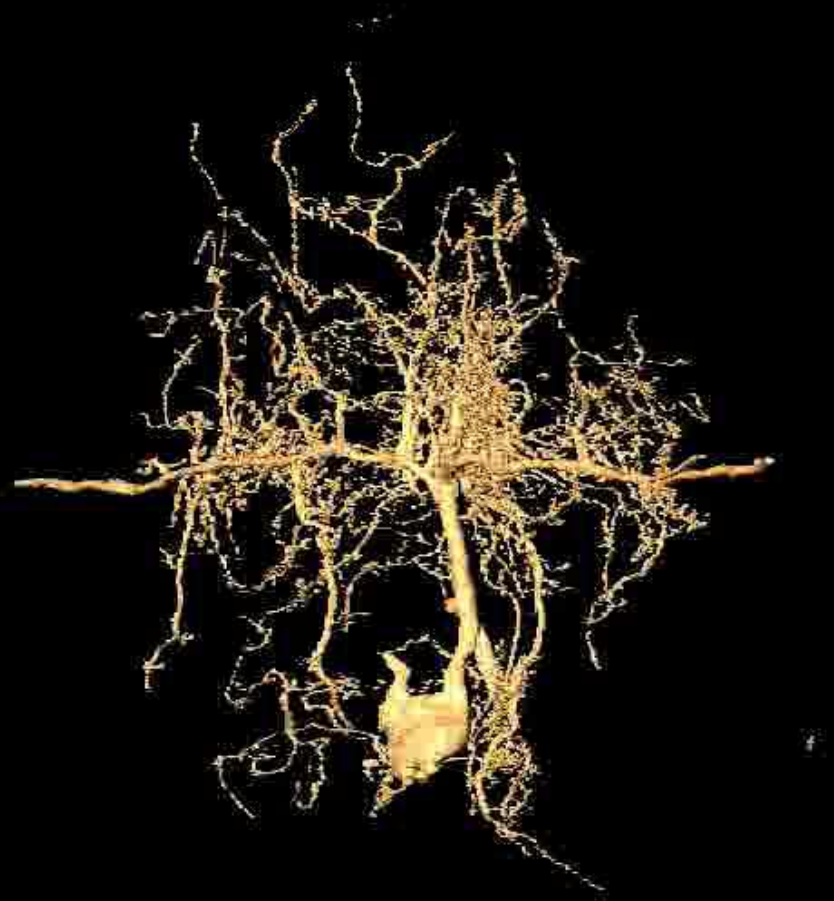


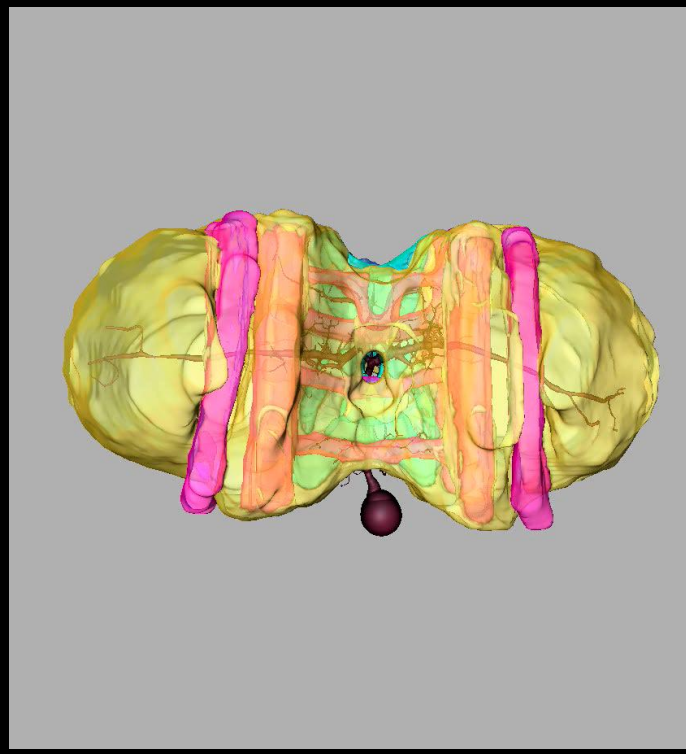


dorsal or ventral unpaired median (DUM or VUM) neurons*

*** most likely a special, autapomorphic feature of insects**

Pflüger & Stevenson (2005), Arthropod Structure & Development 34: 279-296
Stevenson, P. et al., J. Comp. Neurol. 315, 382-397, 1992

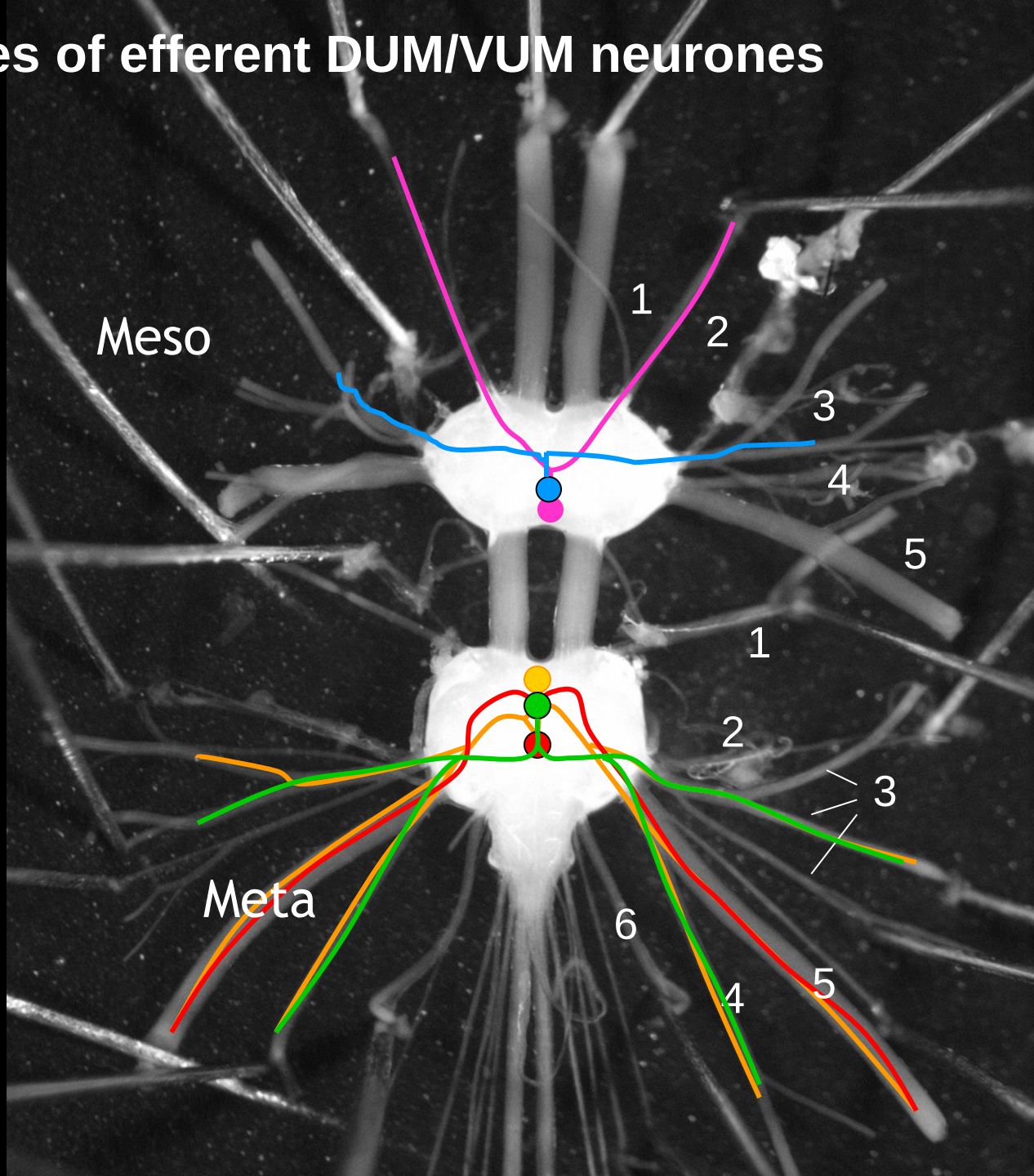


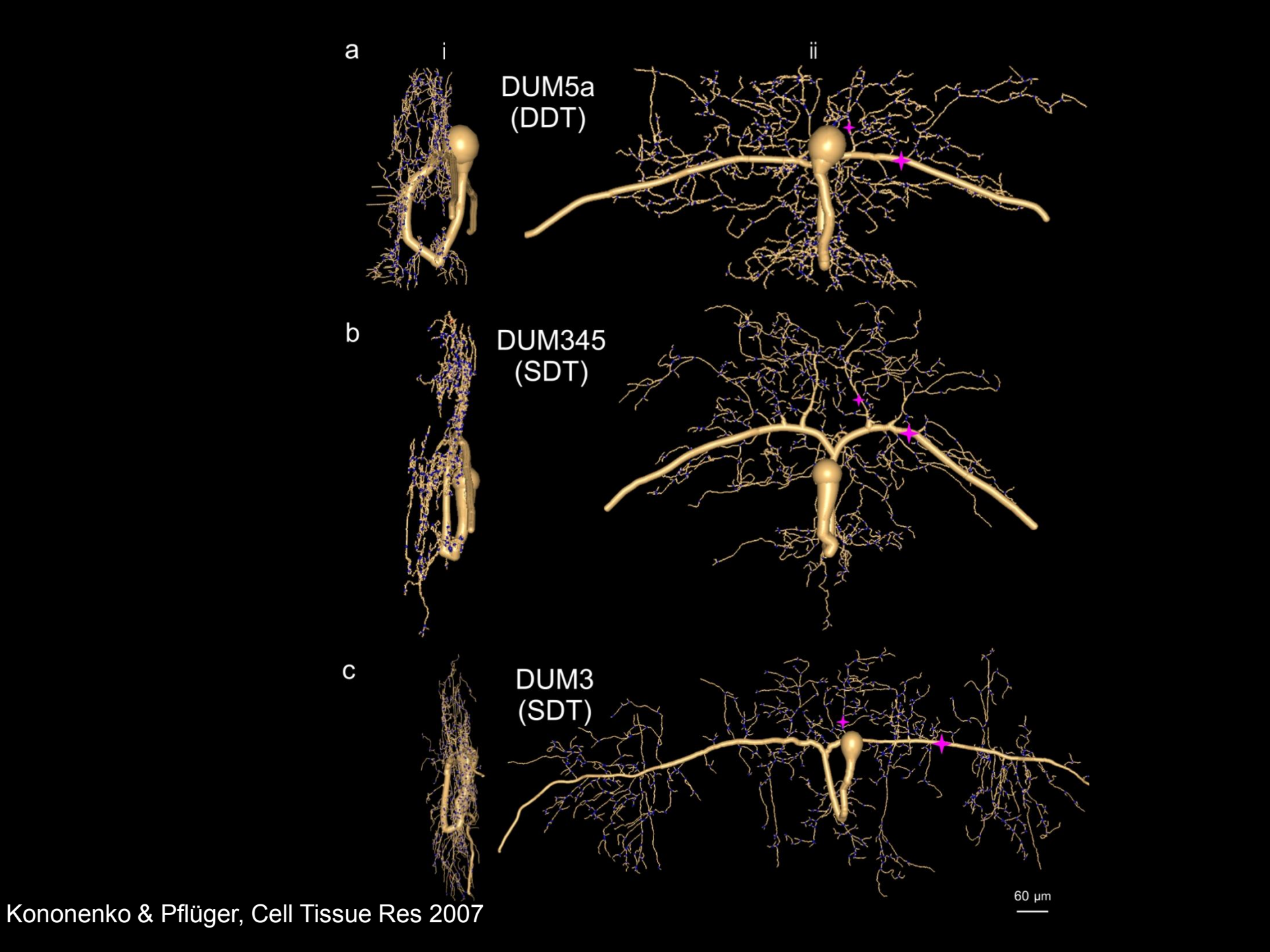


Different types of efferent DUM/VUM neurones

MESO:
2 x DUM1
2 x DUM5s
9 x DUM3,4s
2 x DUM3,4,5
4 x DUM3s

* based on classification system of Watson (1984)





Kononenko & Pflüger, Cell Tissue Res 2007

Thoracic and abdominal ventral cord ganglia

According to their targets and their responses to sensory stimulation the **thoracic efferent unpaired median neurons** (~ 120) can be divided into:

- **A group innervating wing (power) muscles**
  - *mostly inhibited by sensory stimulation, in particular wind from the head*

  (a subgroup may actually innervate bifunctional muscles for both wings and legs and then these neurons are „mildly“ excited by sensory stimuli)

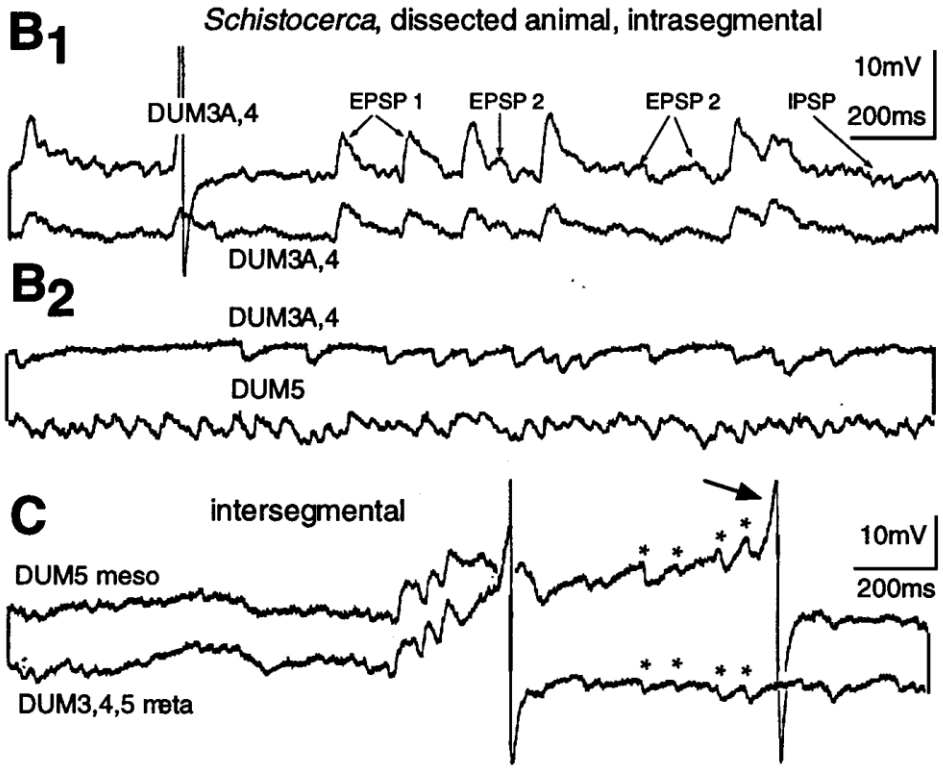
- **A group innervating leg and many other thoracic muscles**
  - *strongly excited by mechanosensory stimuli to the whole body*
  - *with neurohaemal release sites near peripheral nerve branches*

- **A group innervating visceral organs**
  - *heart, genital organs, oviduct, retrocerebral complex*

* In locusts and moths these three different groups are characterized by the existence of **common synaptic inputs** (EPSPs or IPSPs)

* this points to a **common, task specific drive from the head ganglia**

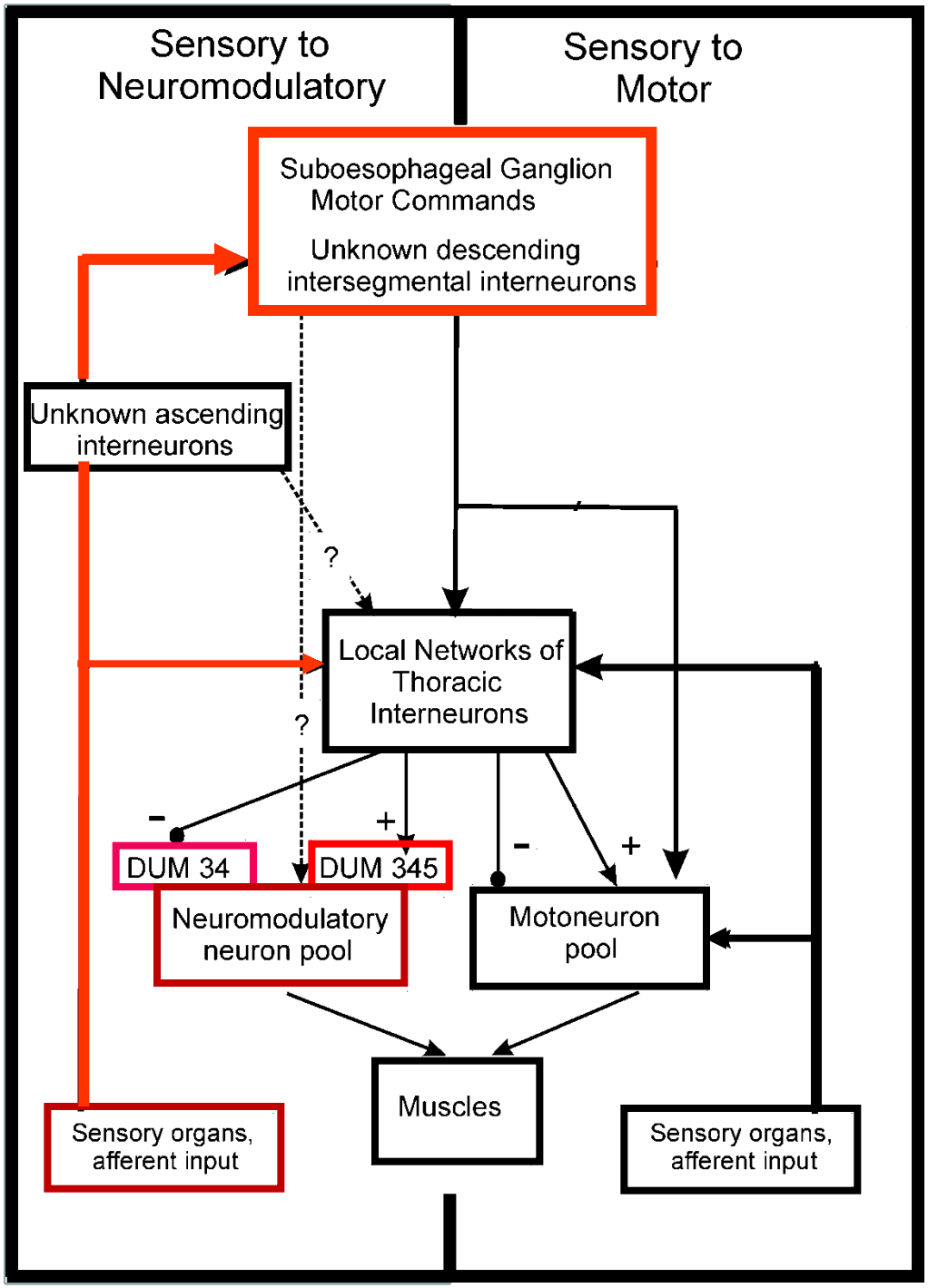
* SOG is the source of these common inputs



* In addition, reflex activation by mechanosensory stimulation is not local but via the SOG

intersegmental sensory pathways to SOG

polysynaptic local pathways



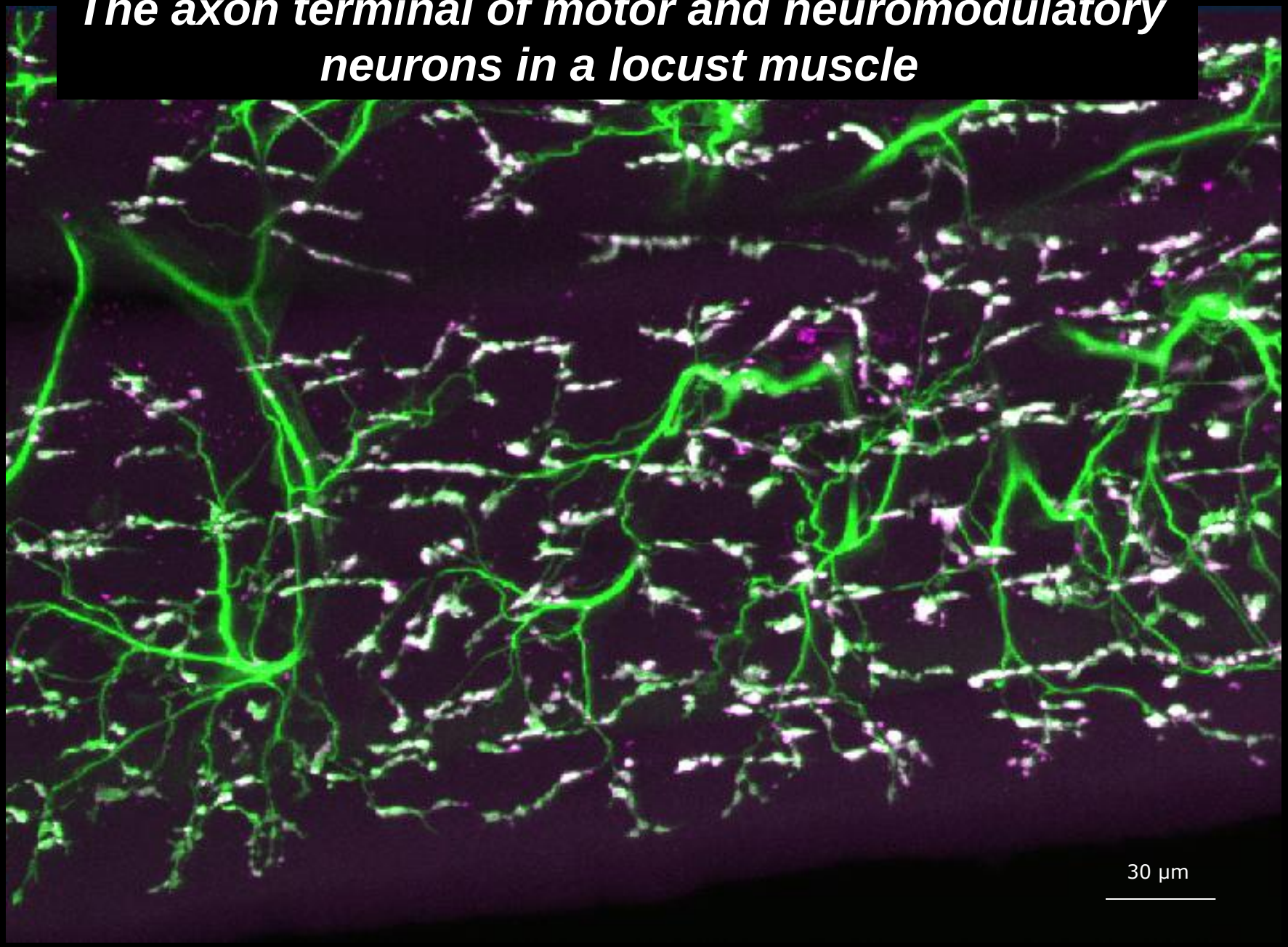
polysynaptic local reflexes (Burrows 1996)

monosynaptic local reflexes (Burrows 1996)

from:
Field, Duch and Pflüger, 2007, J Insect Physiol

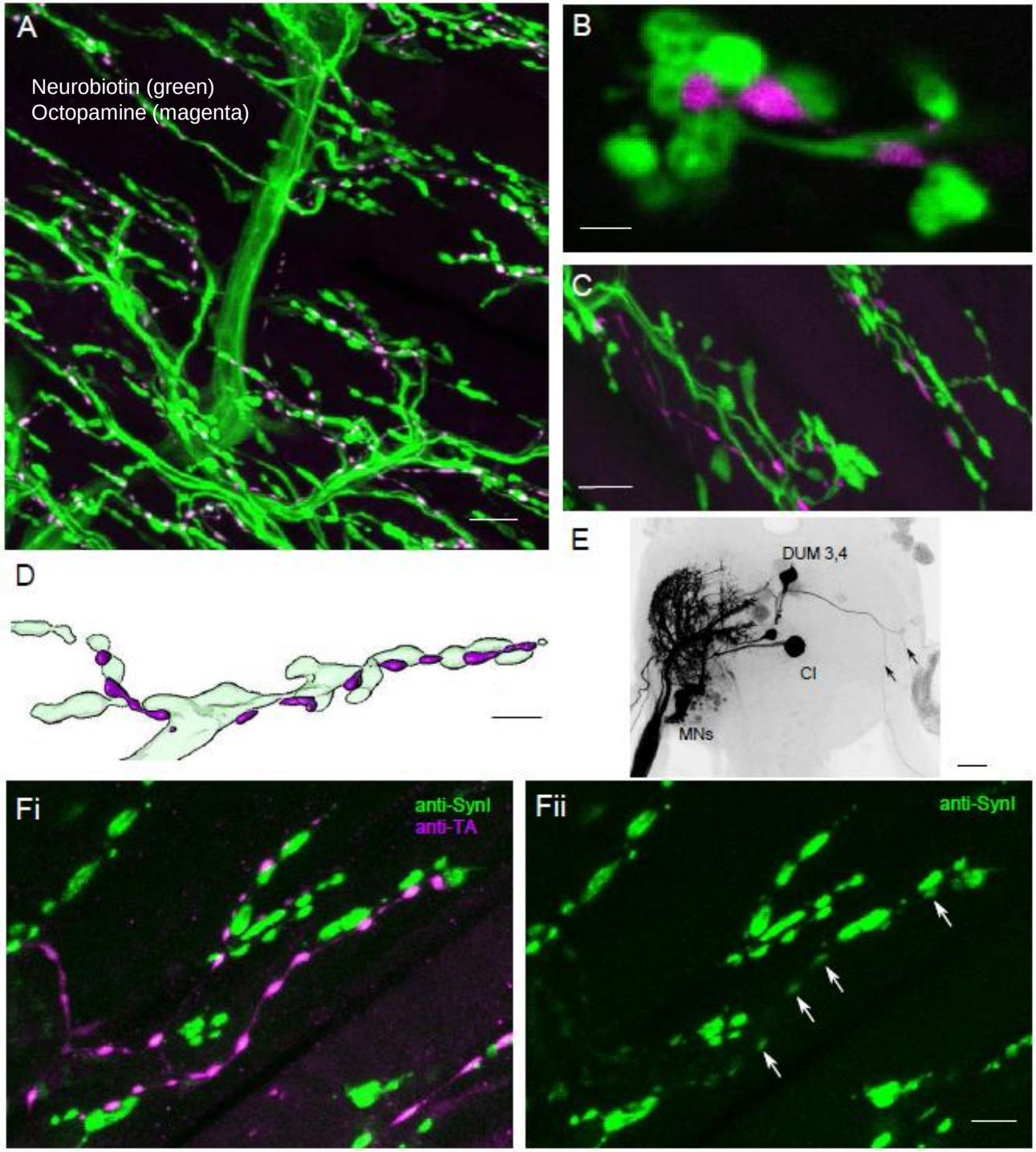
The peripheral aspects of DUM/VUM neurons

The axon terminal of motor and neuromodulatory neurons in a locust muscle

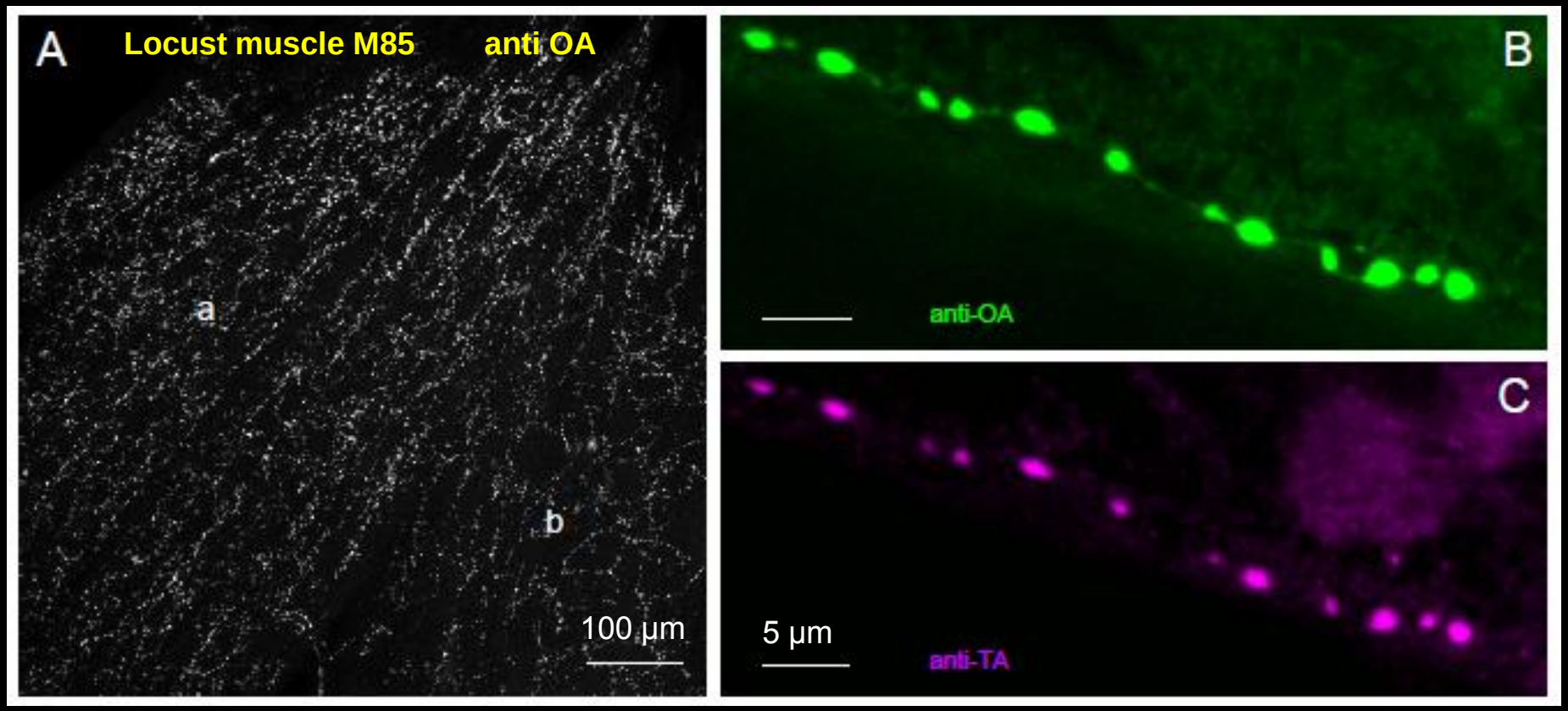


PhD-thesis Bettina Stocker

Neurobiotin Synapsin projection view (z=27 µm)



PhD-thesis
Bettina Stocker
FU Berlin 2011



z = 30 µm

z = 11 µm

In locusts, all axonal terminals of a DUM3,4,5 neuron exhibit both tyramine-IR and octopamine-IR.

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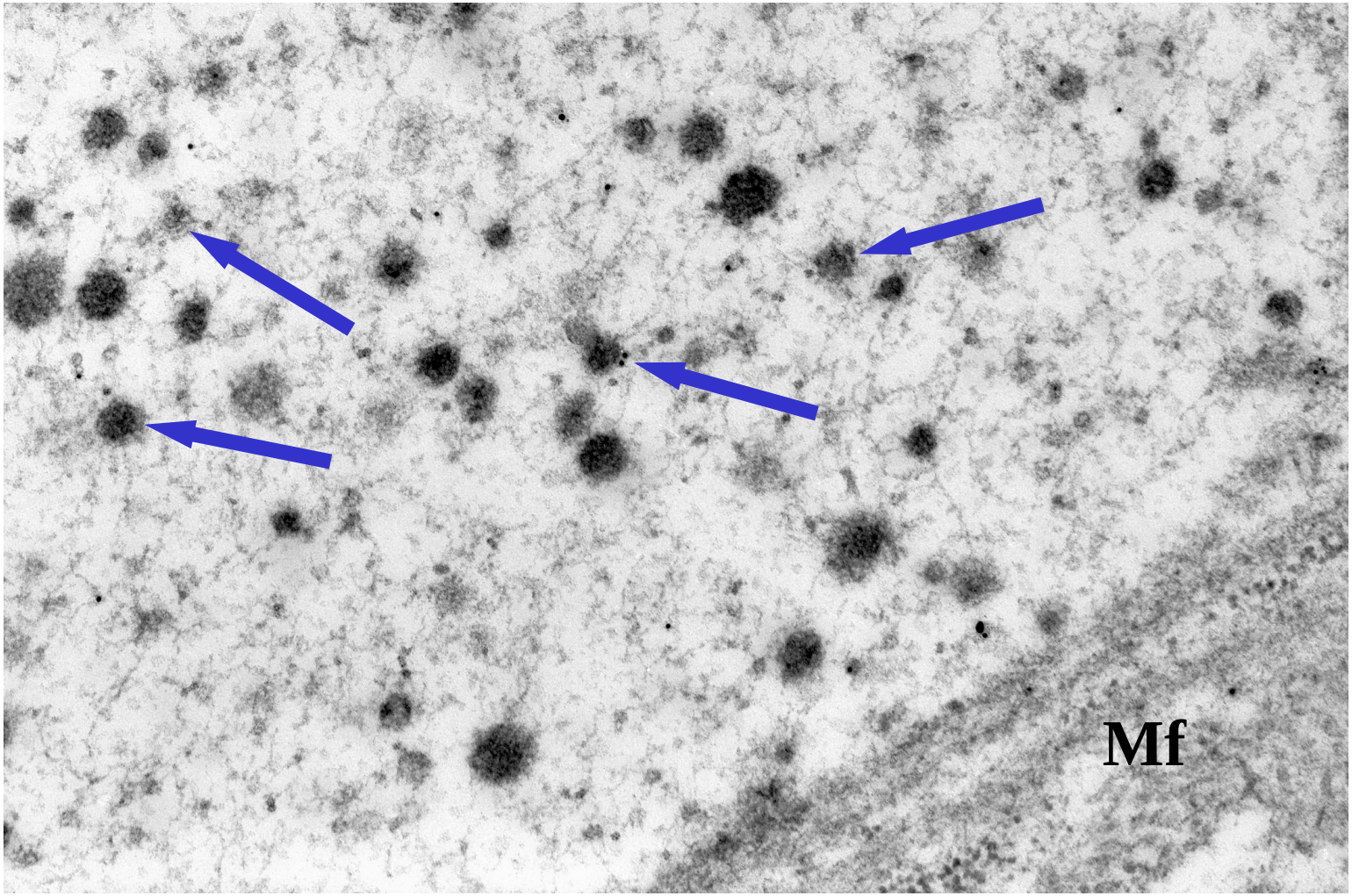
Octopamine-immunoreactivity in round dense core vesicles (blue arrows) of a nervous terminal on the surface of a locust visceral muscle (Mf, muscle fiber of oviduct). (from Biserova and Pflüger, unpublished)

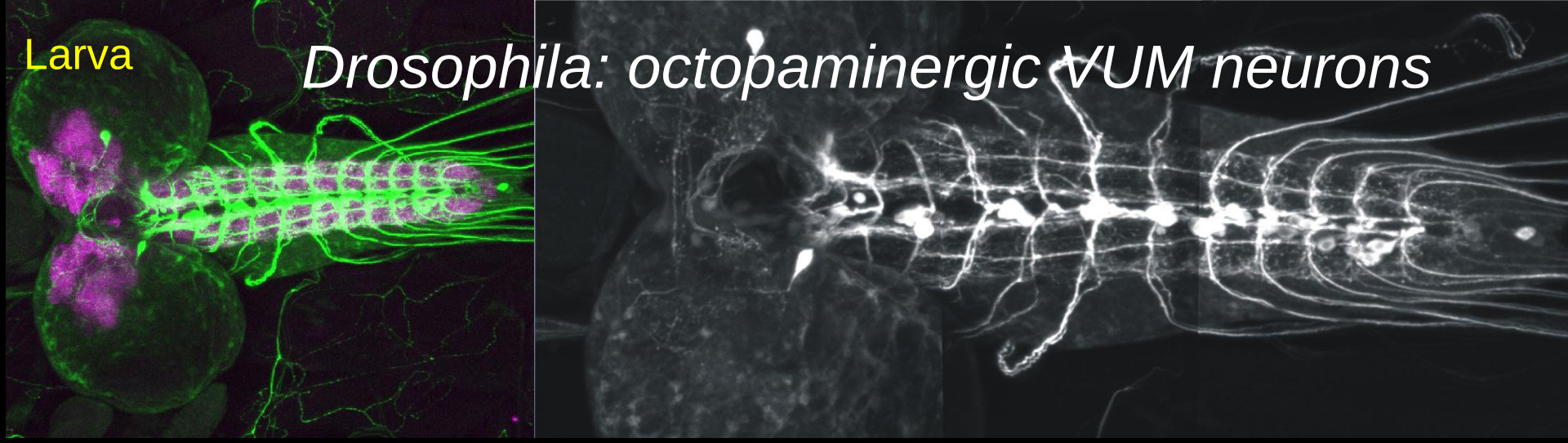


….similar findings apply to the fruit fly (*Drosophila melanogaster*)

Source: http://500px.com/photo/6350247

http://www.biologycorner.com/fruitfly/fruit_fly_pic.jpg

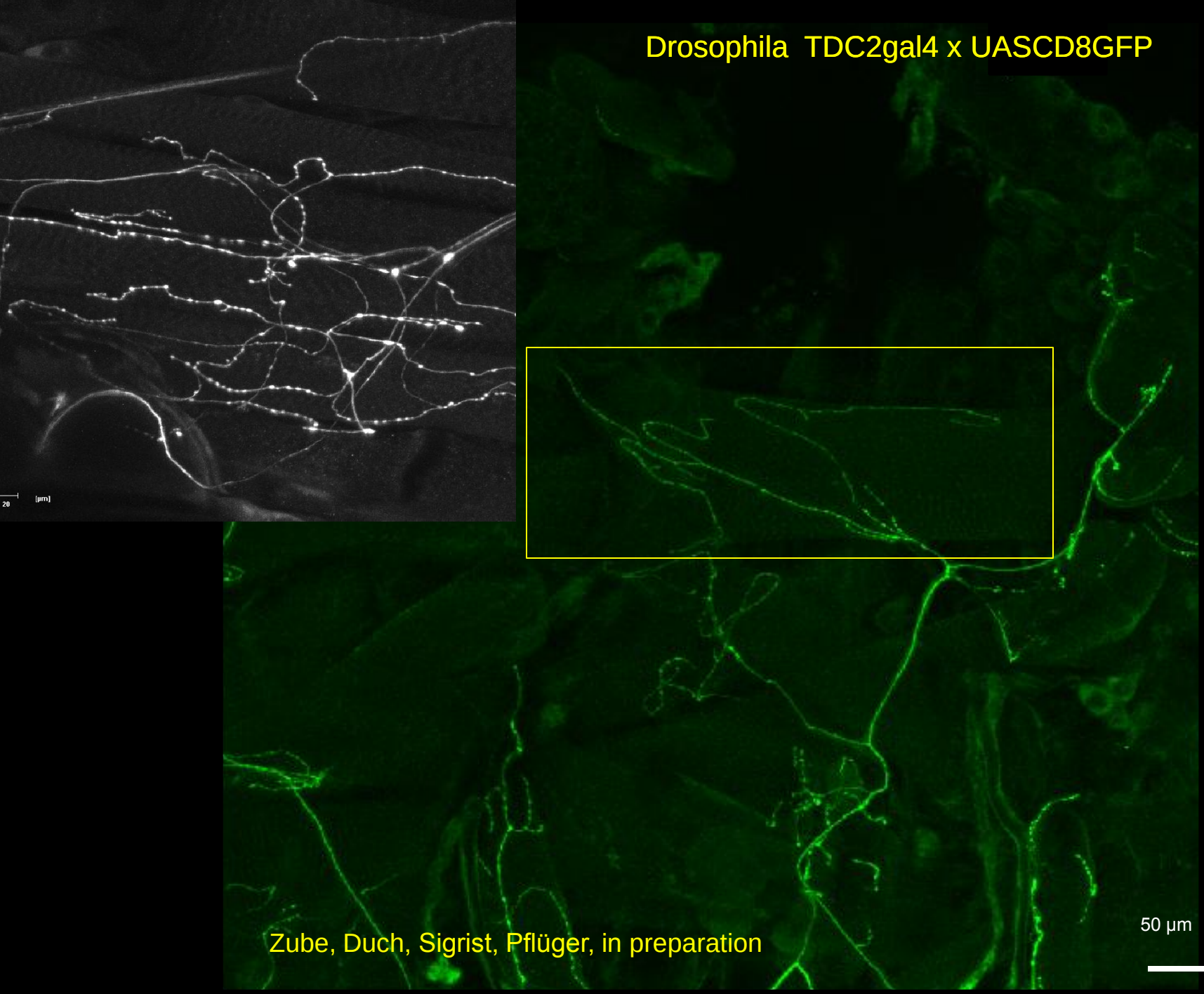
Drosophila: octopaminergic VUM neurons



a-HRP a-GFP a-NC82 merged

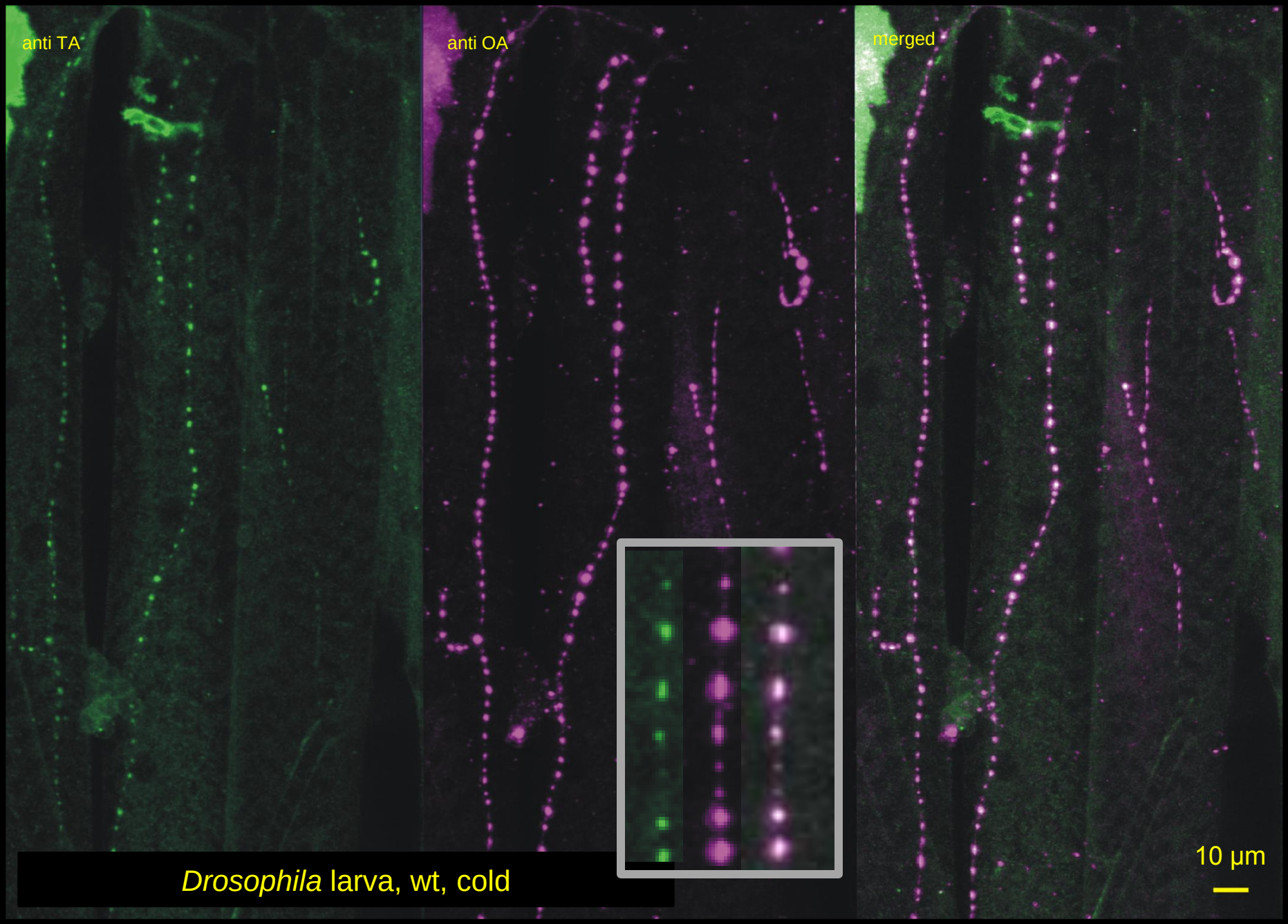
adult *Drosophila* TDC2-gal4 x UAS-CD8-GFP

Pflüger, Zube, Sigrist, Duch unpublished



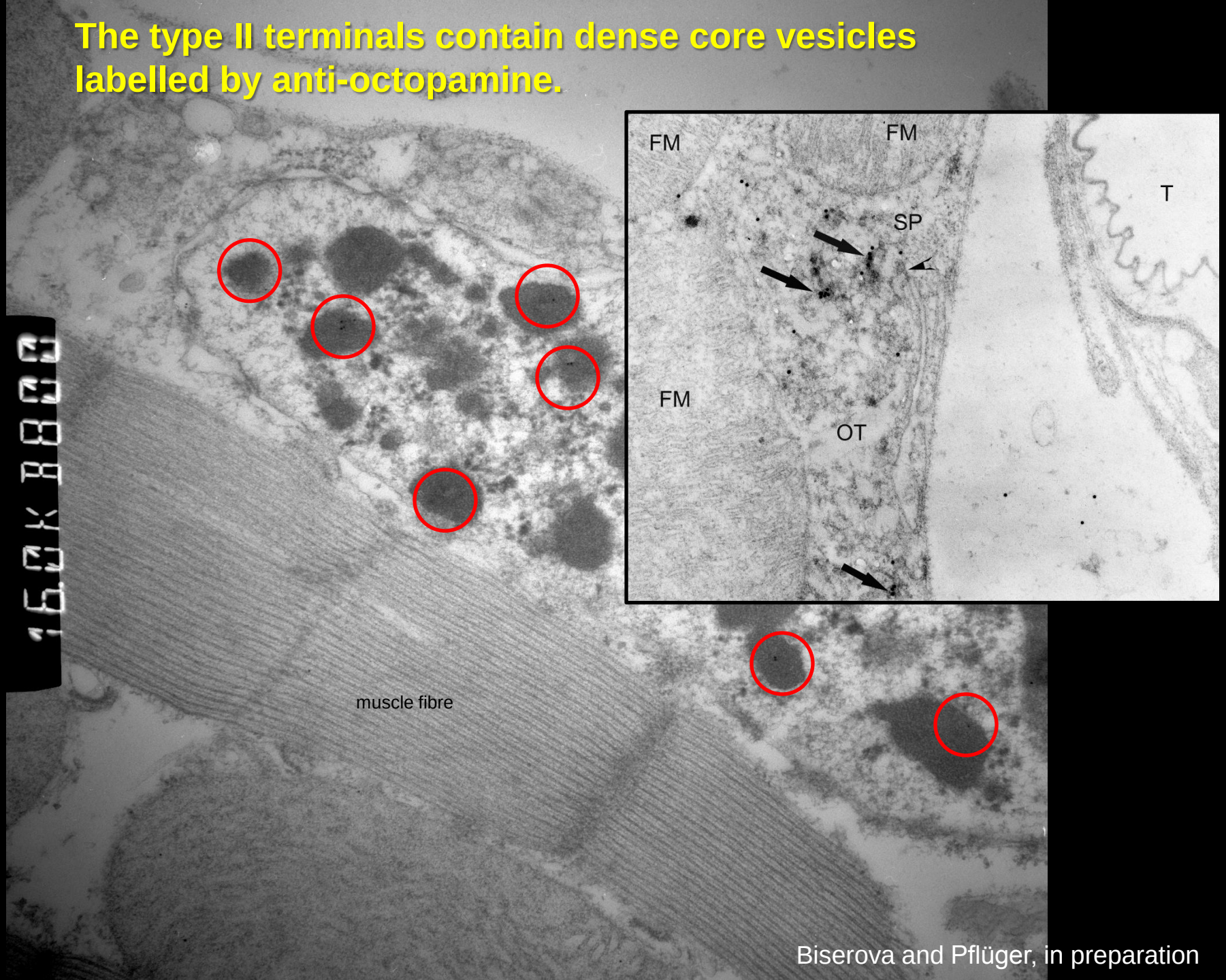
Zube, Duch, Sigrist, Pflüger, in preparation

The type II terminals are tyraminergic/octopaminergic

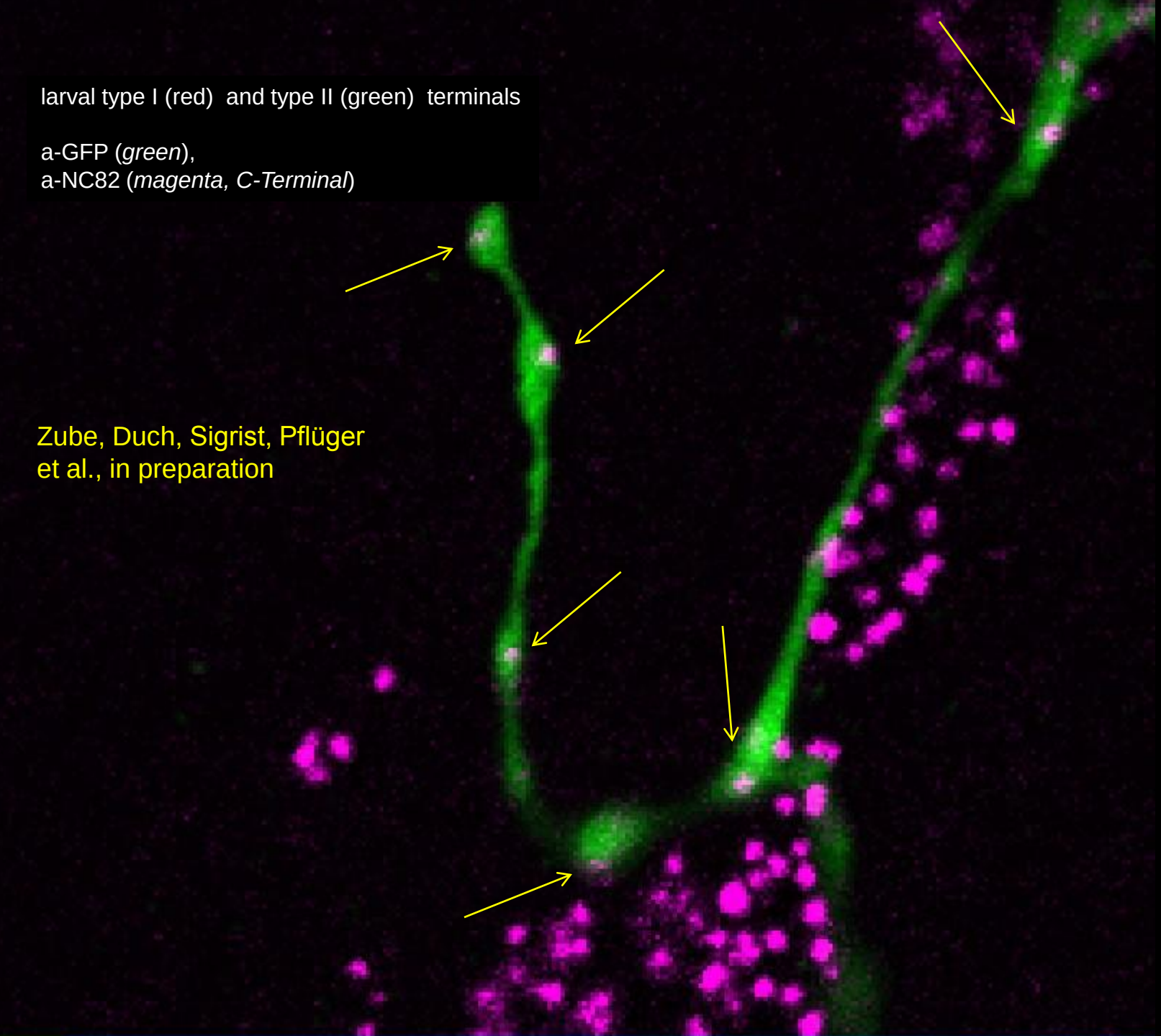


Drosophila larva, wt, cold

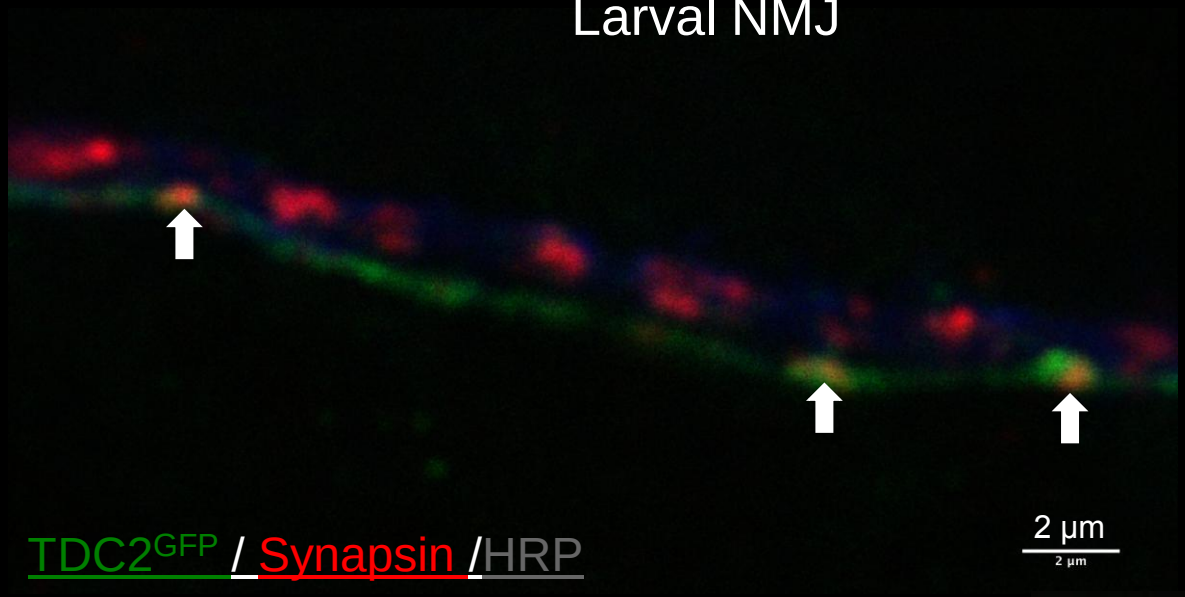
The type II terminals contain dense core vesicles labelled by anti-octopamine.



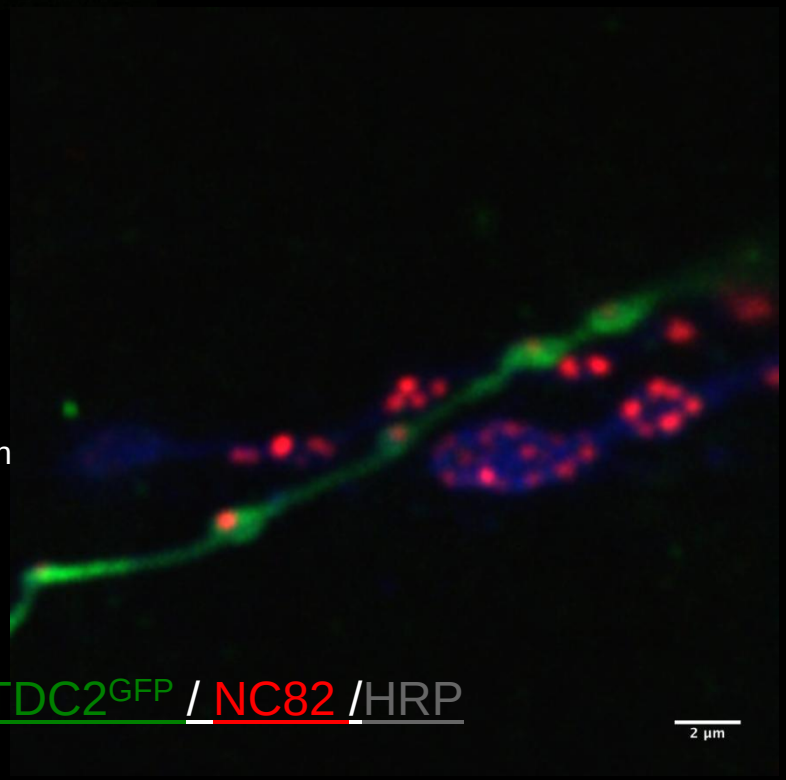
Biserova and Pflüger, in preparation


larval type I (red) and type II (green) terminals
a-GFP (*green*),
a-NC82 (*magenta, C-Terminal*)
Zube, Duch, Sigrist, Pflüger
et al., in preparation

Larval NMJ



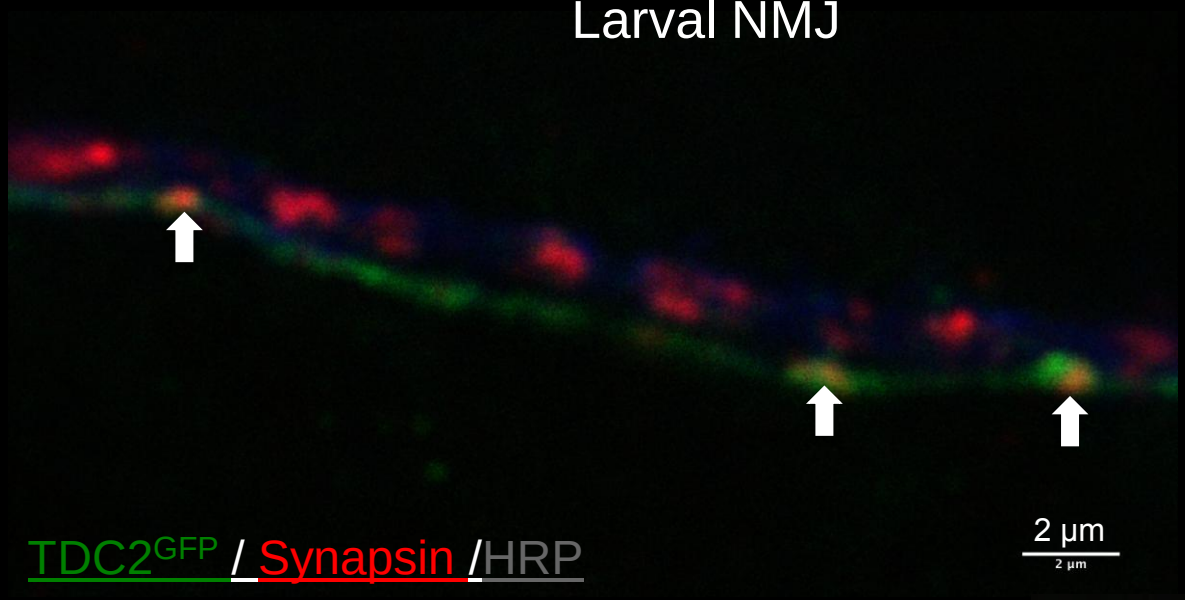
TDC2^{GFP} / Synapsin /HRP



TDC2^{GFP} / NC82 /HRP

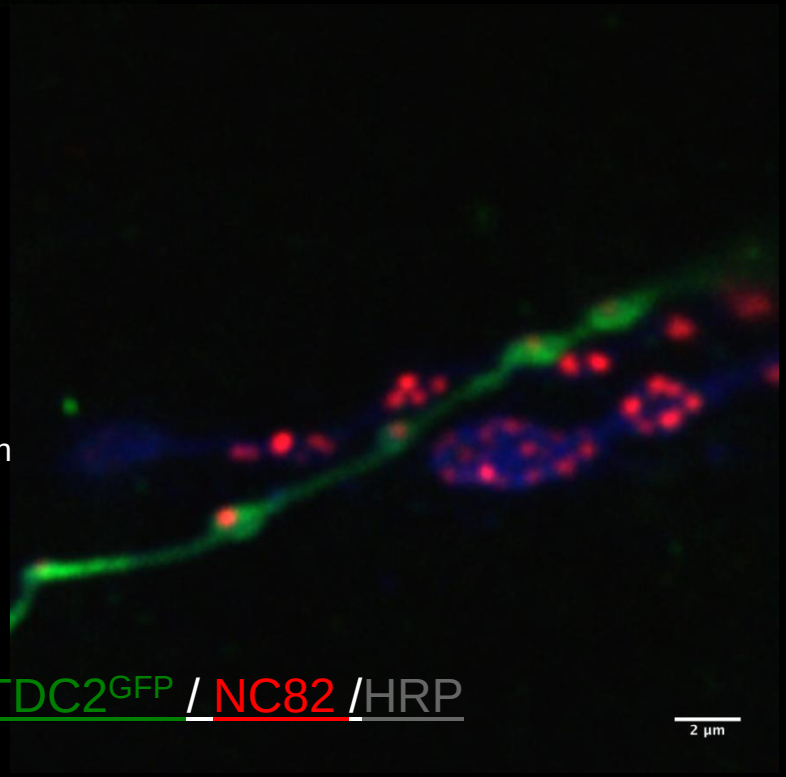
Zube, Duch, Sigrist, Pflüger, et al., in preparation

Larval NMJ



TDC2^{GFP} / Synapsin /HRP

The type II terminals seem to use similar synaptic proteins for vesicular release as the „classical“ type I neuromuscular junctions (both in larvae and adults)



TDC2^{GFP} / NC82 /HRP

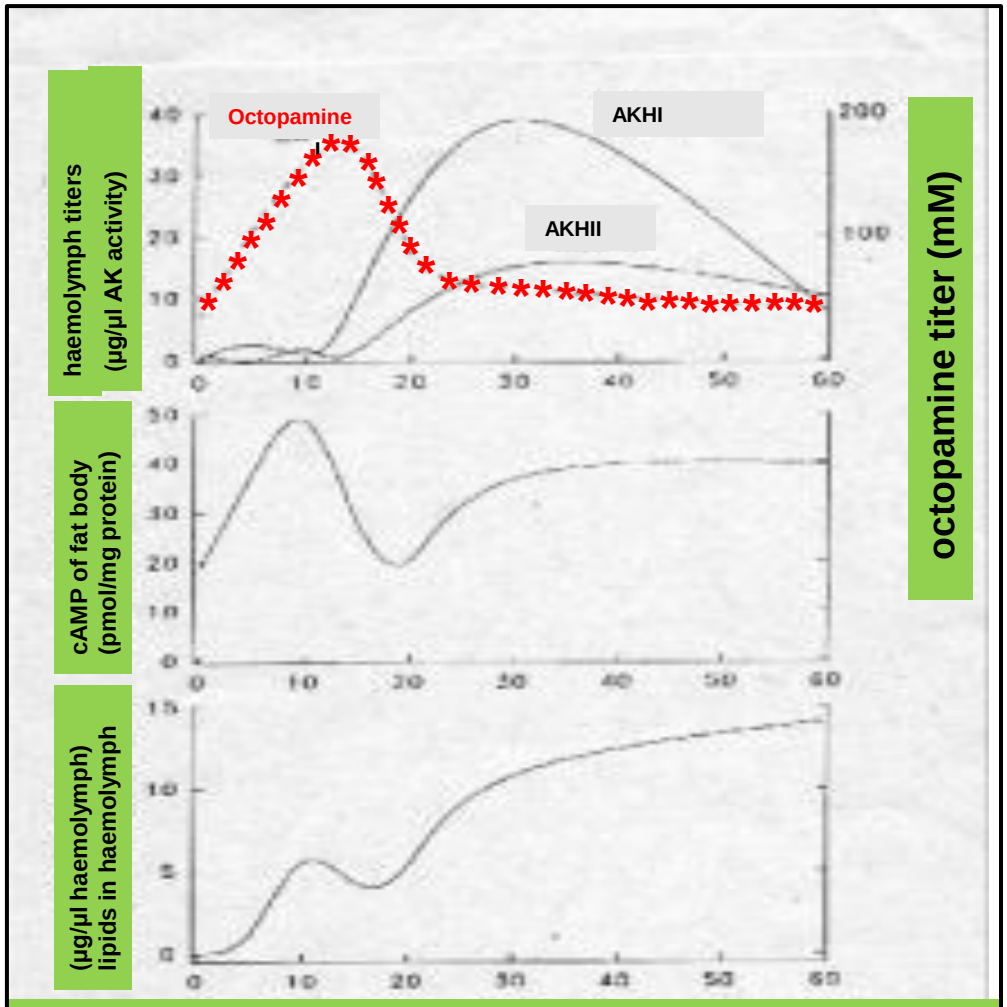
Zube, Duch, Sigrist, Pflüger, et al., in preparation

Neurohormone
versus
Neuromodulator

The neurohormonal role of octopamine

- haemolymph concentration increased when hungry, when aggressive, or in the beginning of flight or in other, energy demanding behaviours

Experimental data



data from Orchard, Ramirez et al.

The neurohormonal role of octopamine

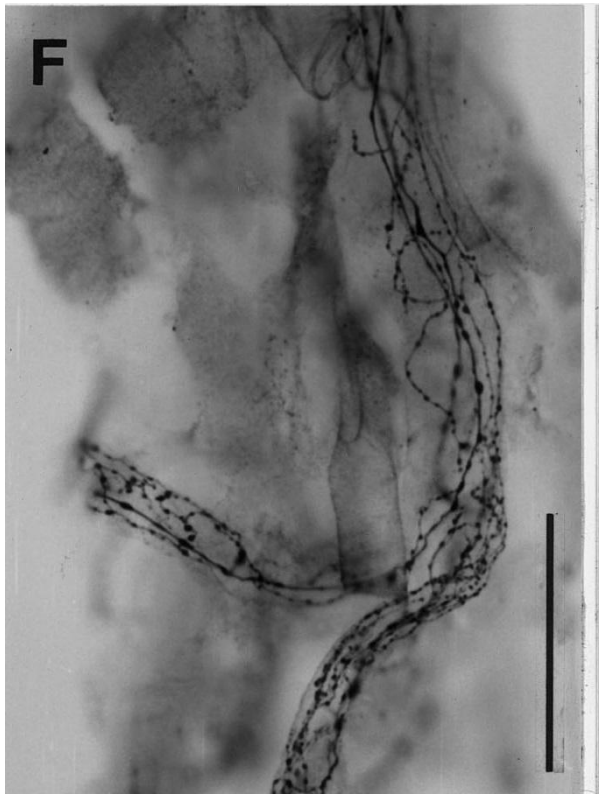
- haemolymph concentration increased when hungry, when aggressive, or in the beginning of flight or in other, energy demanding behaviours

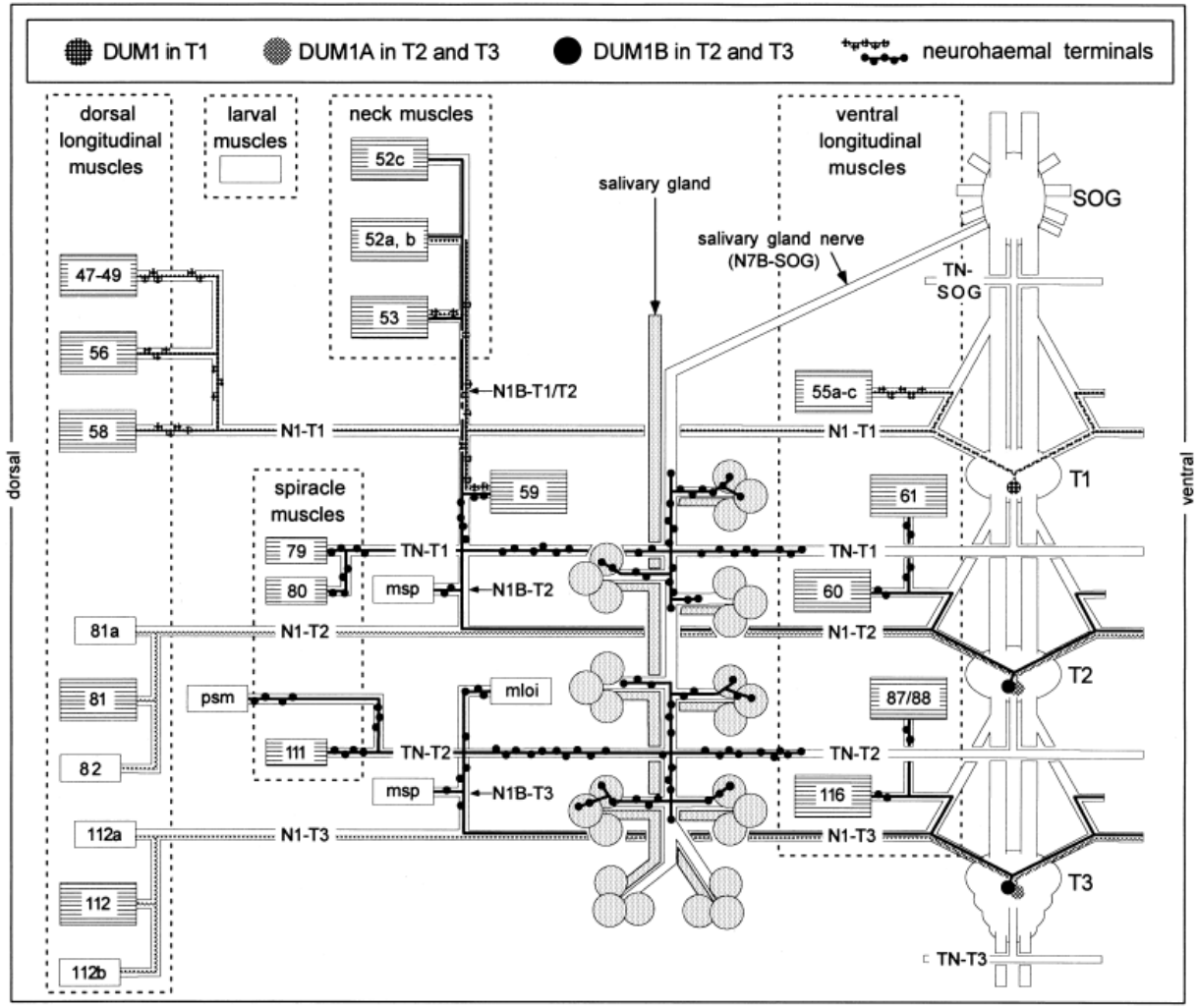
- hyperglycaemic action:
  - trehalose mobilized from fat body
  - fatty acids mobilized from fat body by breaking down lipids

- OA acts on cells of the fat body and, perhaps, on tracheole cells to also promote gas exchange

- OA concentration increased for about 10-15 min, then AKHs (*adipokinetic hormones*) take over and OA remains at a lower level

Which neurons are responsible for the release of OA into the haemolymph?



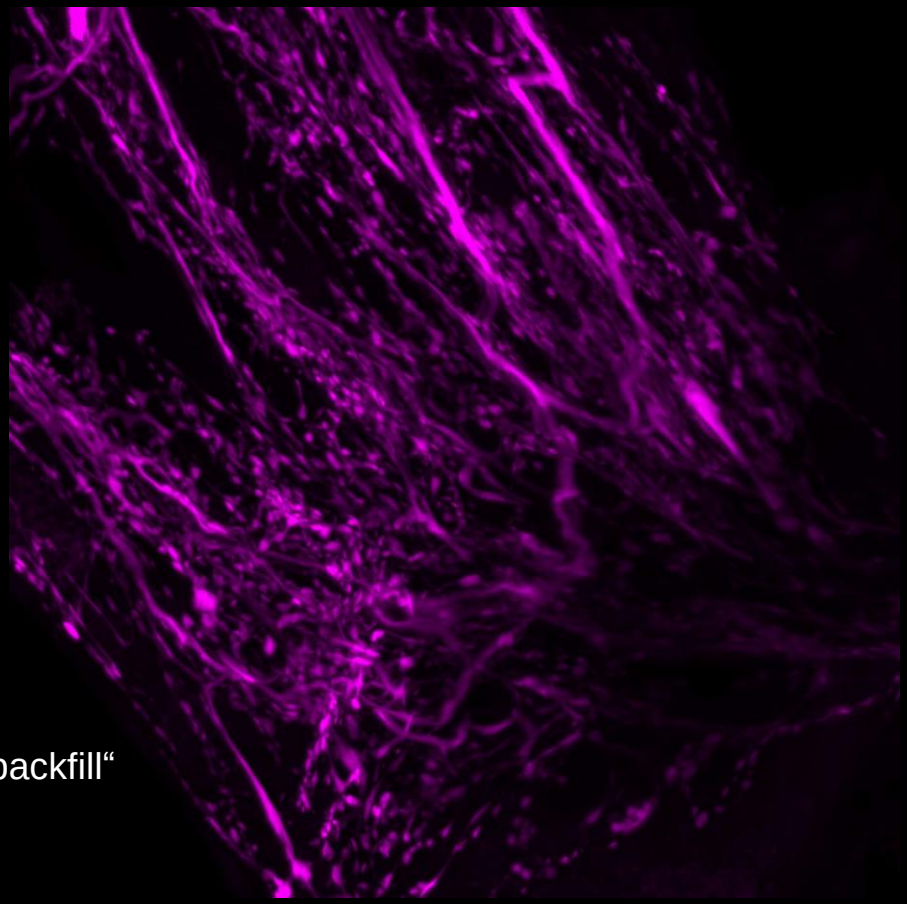


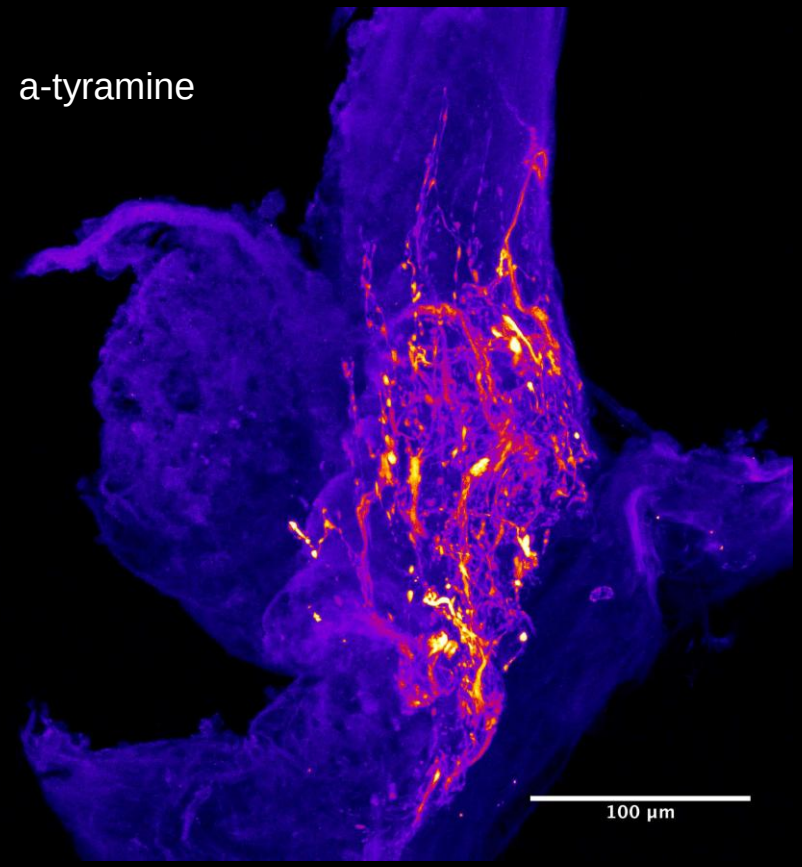
Neurohaemal release sites of DUM neurones

Bräunig, Cell Tissue Res 1997

* Particular DUM neurons may possess additional neurohaemal release sites in addition to their specific targets

In addition, specific release sites such as antennal heart*
(*innervated by tyraminergic/octopaminergic unpaired median neuron of the suboesophageal ganglion*)





Many studies by Penzlin, Pass et al., and Master thesis Victoria Antemann, FU Berlin, 2014

Summary

Peripheral modulatory functions:

1) approx. 125 tyraminergic/octopaminergic neurons possess peripherally projecting axons and innervate all skeletal and visceral muscles, and salivary glands, retrocerebral complex and proprioceptive sense organs

2) **Modulate catabolism** of target tissues in „*short terms*“
   - *boosting glycolysis*, *may also provide a switch between carbohydrate and lipid metabolism*
   - **wing power muscles**
     (DUM neurons active at rest, inhibited during flight, production of a metabolic signaling substance (*F2,6BP*) required for boosting glycolysis) *lipid metabolism*
   - **leg/thoracic muscles**
     (DUM neurons always active during movement, boosting glycolysis)

3) Also **modulate efficacy of neuromuscular transmission**
   - small increase of twitch
   - increase of relaxation rate (a significant parameter to enable faster movements)
   - prevention of catch effects

4) Additional systemic release of OA may be responsible for neurohormone-actions such as hyperglycaemic action (*releasing trehalose and free fatty acids from fat body and, perhaps, actually also boosting* CO_2/O_2*-exchange*)

Visceral muscle (for example oviduct or „accessory hearts“)

- Inhibition of all myogenic contractions (*which many visceral tissues generate*)

….in shorter words:

Prepare skeletal and visceral muscles and other target organs for soon to come dynamic action such as locomotion or flight.

Duch and Pflüger, J Comp Physiol A, 1999
Mentel et al., J. Neurosci., 2003
Blau und Wegener, 1994
Blau, Wegener and Candy, Insect Biochem Molec Biol 1994
Wicher, Endocrine, Metabolic and Immune disorders – drug targets, 2007
Koon et al., Nature Neurosci, 2011

A remarkable
plasticity of the axonal terminals
of **efferent octopaminergic unpaired median neurons** was observed
in *Drosophila* larvae:

Behavioral observation:

- Increase in locomotion speed after food deprivation

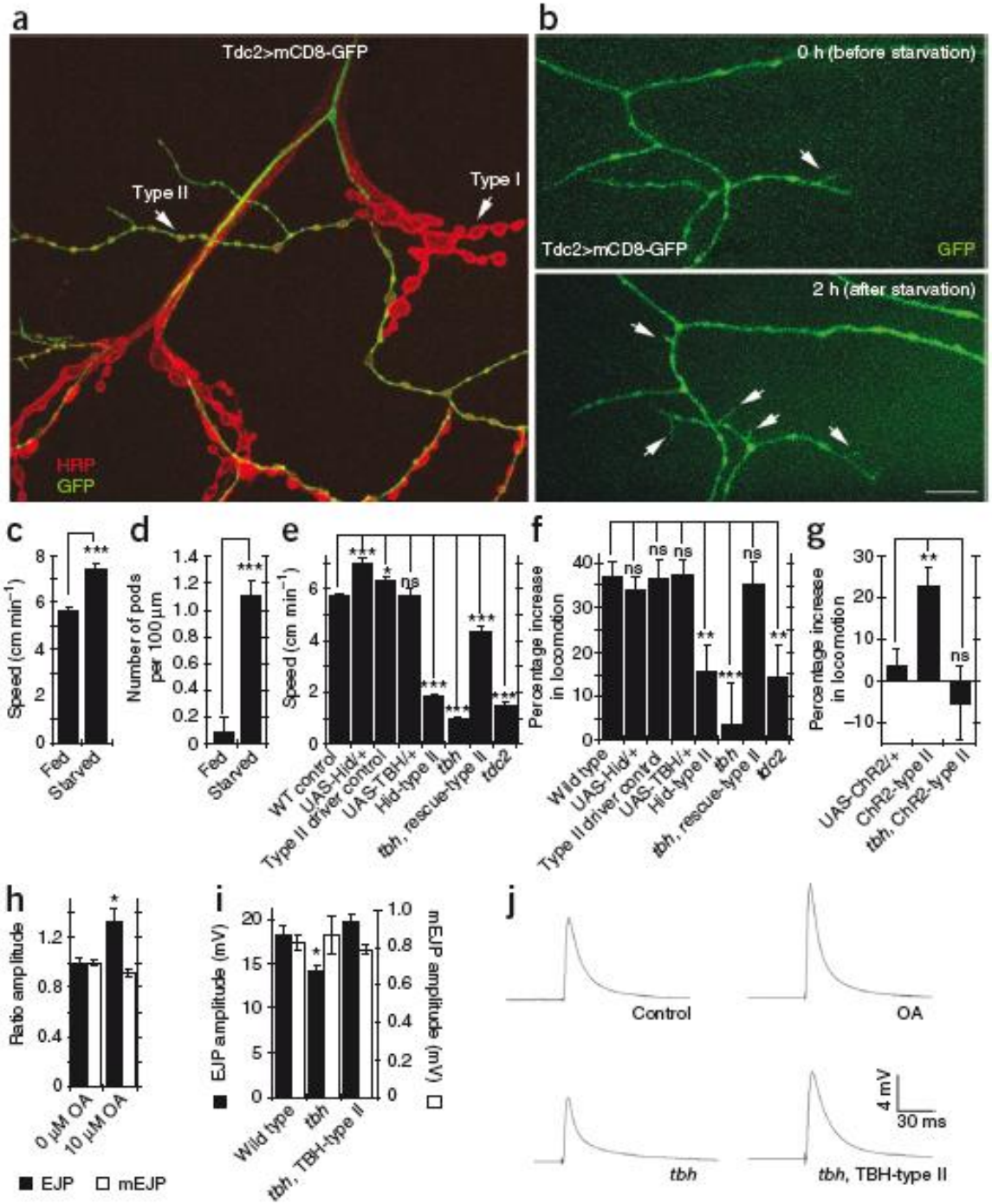
- Activity- and octopamine-dependent extension of axonal branches of octopaminergic neurones on muscle („synaptopods“)

- Growth of octopaminergic-axons required a cAMP- and CREB-dependent positive-feedback mechanism dependent on Octß2Rs (autoreceptors)

- Octopamine neurons also control expansion of excitatory glutamatergic axon terminals (type I) through Octß2Rs on these terminals

* This (growth) process is inhibited by Octß1R, via inhibition of cAMP.

(results from Koon et al. (2011), Nature Neuroscience, 14:190-201 and Koon and Budnik (2012), J Neurosci 32(18):6312-22, in Drosophila larvae)



Formation of „synaptopods“

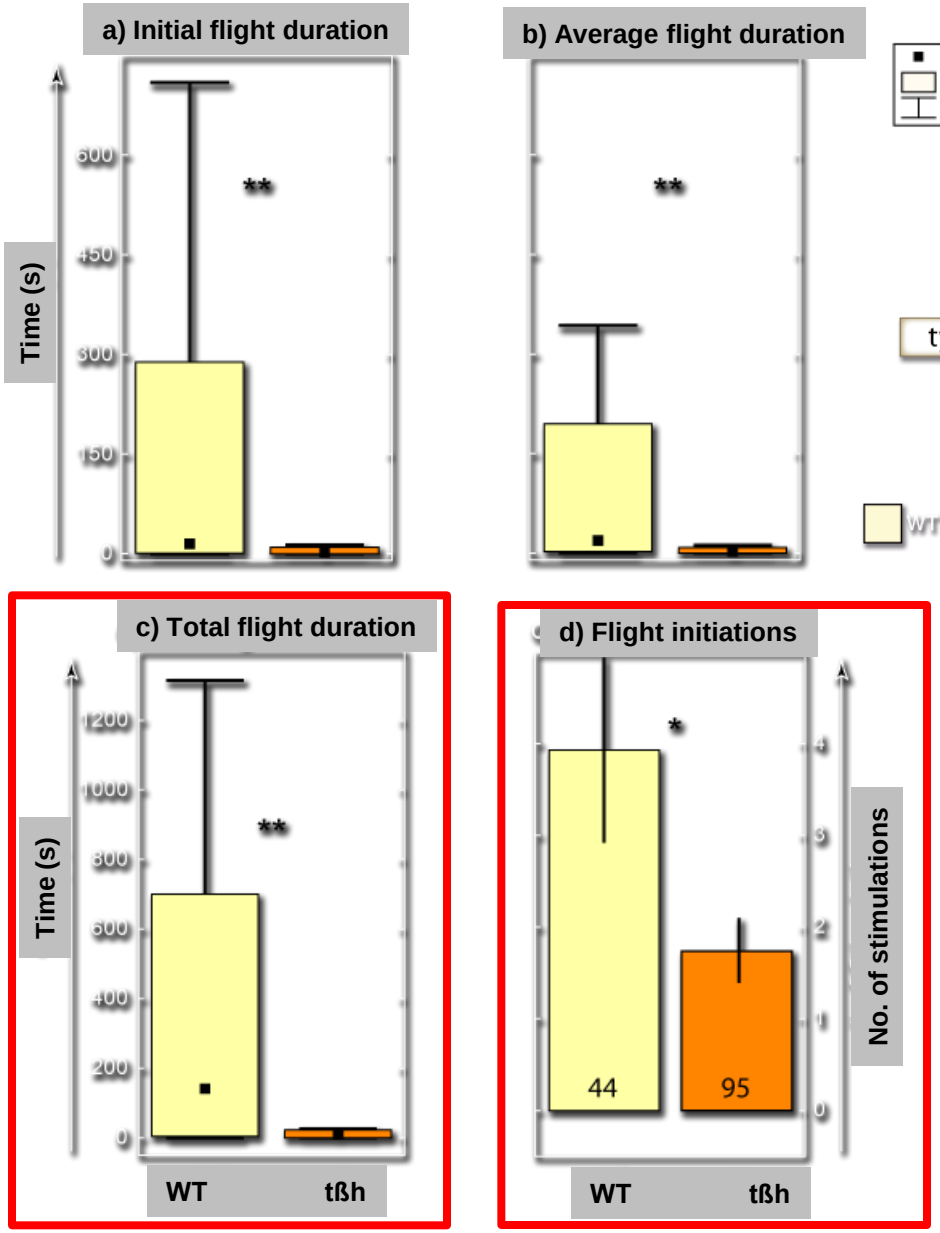
Koon et al. (2011)
Nature Neuroscience 14:190-201

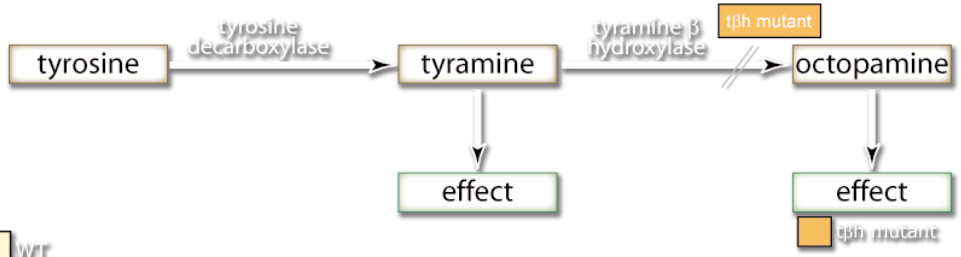
Courtesy Björn Brembs

How does a mutant fruit fly*, *Drosophila melanogaster*, which cannot synthesize Octopamine, fly ?

- *Drosophila* tßh-mutant cannot produce octopamine (Monastirioti 1995) (tßh-mutant kindly supplied by H. Scholz, Würzburg, now Köln)
- *Drosophila* uses carbohydrates („sugars“) as fuel not lipids

Severe flight performance deficits in flies without octopamine

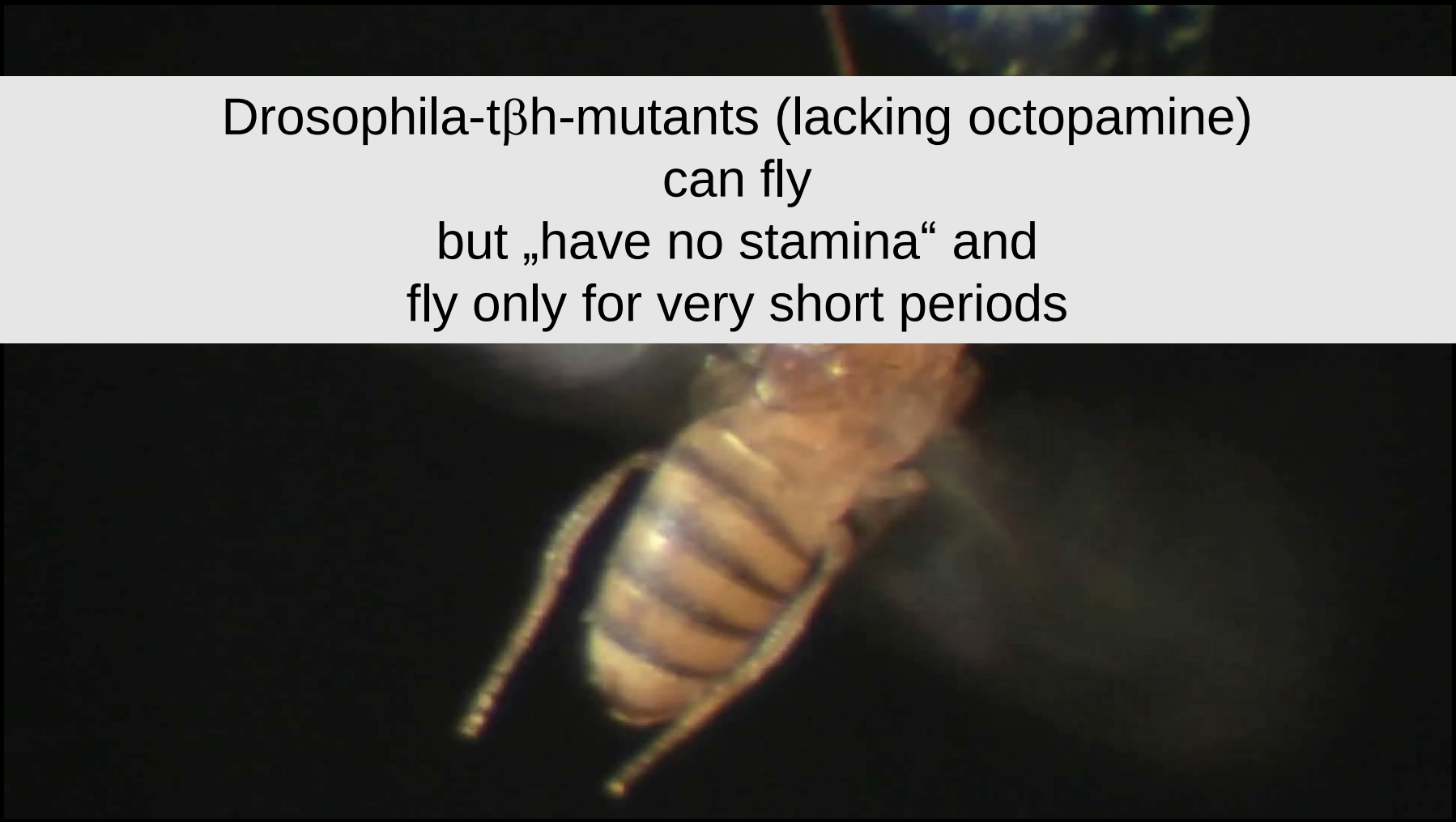




* The effects of tßh-mutants can be rescued
(i) by either feeding OA,
(ii) or also feeding yohimbine
(thus blocking TA-receptors)
(iii) by genetic methods

Brembs, Christiansen, Pflüger, Duch (2007) J Neurosci 27:11122-11131

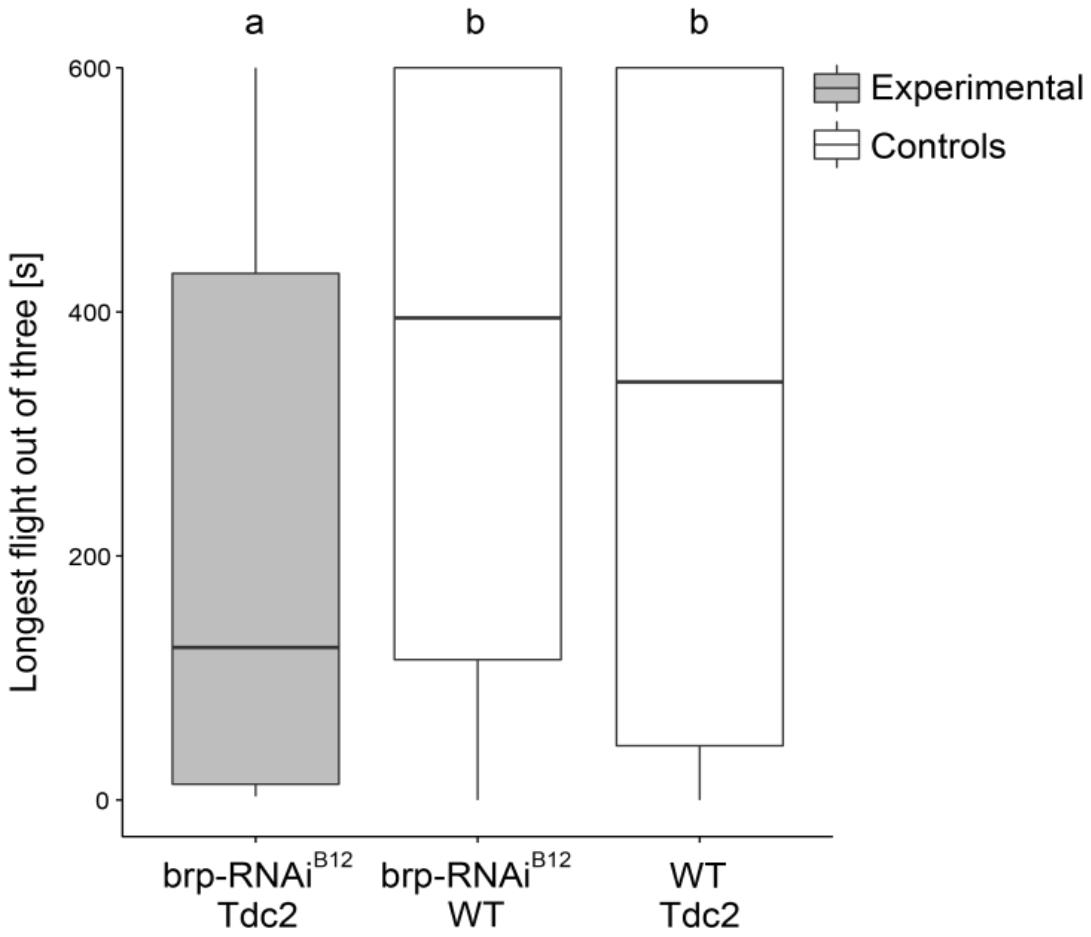
Drosophila-tβh-mutants (lacking octopamine)
can fly
but „have no stamina“ and
fly only for very short periods



Is the release of octopamine from octopaminergic axonal terminals necessary for proper insect flight ?

* release mechanisms similar to those of neuromuscular synapses (*the same synaptic proteins for vesicle release have been found*)

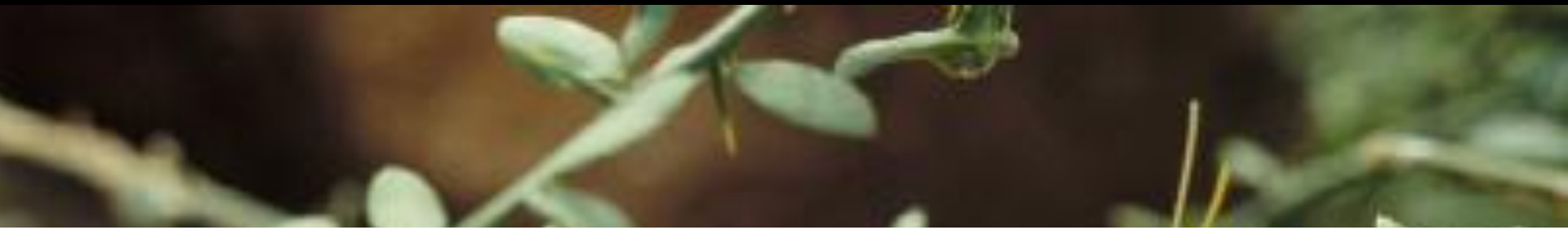
* Therefore, blocking synaptic release (by inhibiting the *Bruchpilot(brp)-gene)* should interfere with the flight performance of *Drosophila*



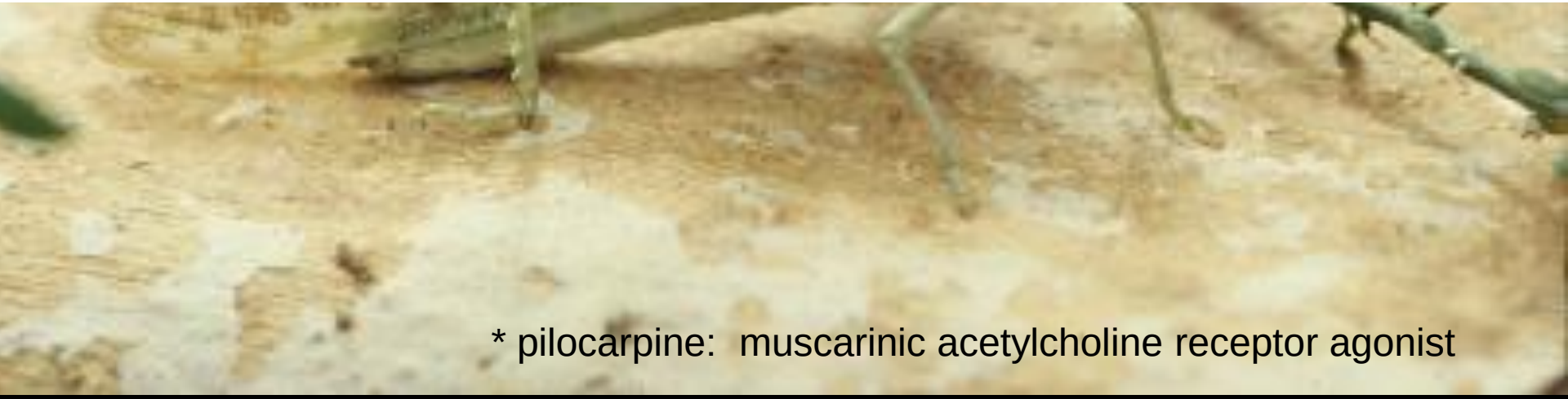
Expression of bruchpilot-RNAi in octopaminergic and tyraminergic cells led to a decreased flight duration.

unpublished data Damrau, Colombes and Pflüger, 2014

Tyramine and Octopamine
are modulators of central pattern generators

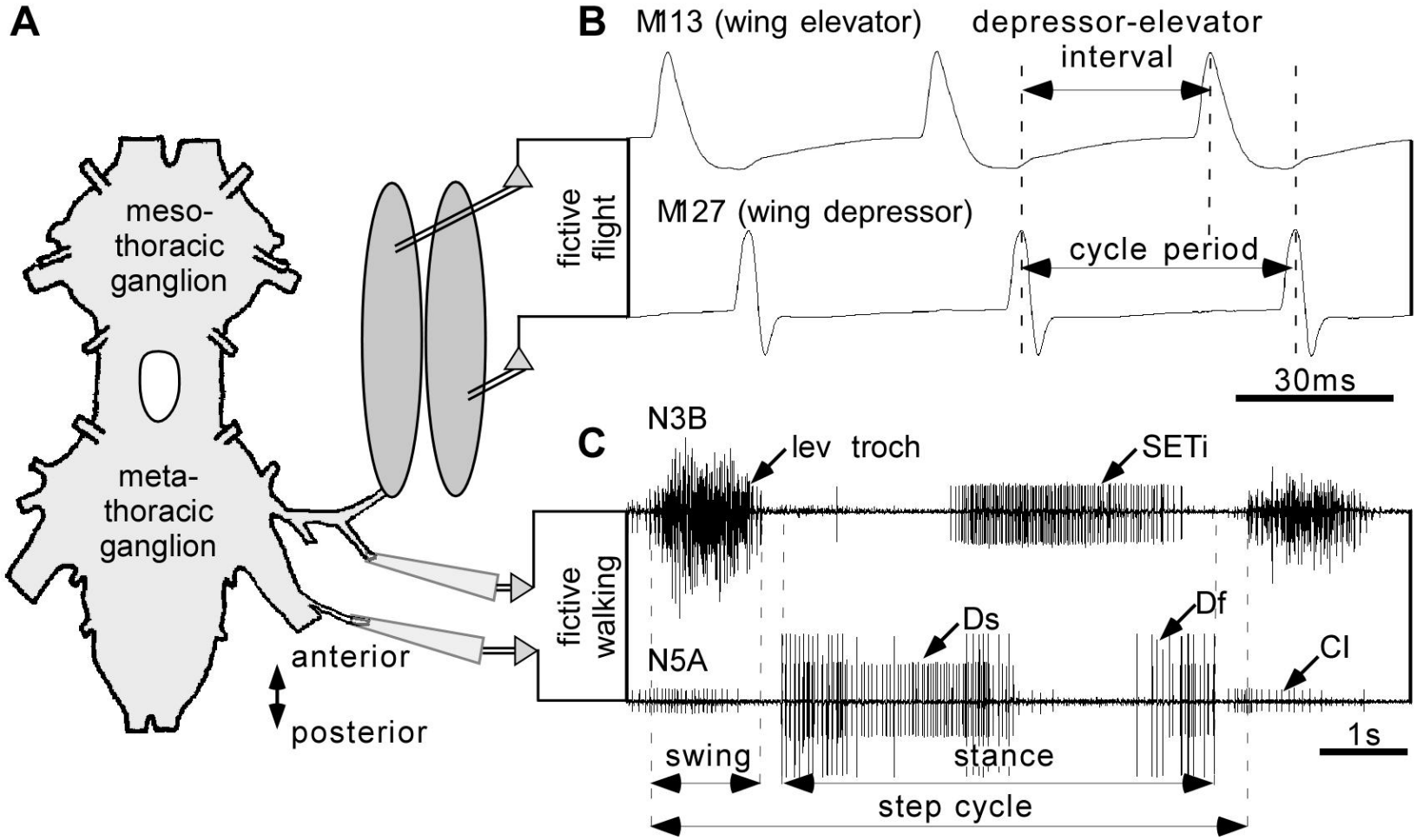


Effects of pilocarpine*, tyramine and/or octopamine on centrally elicited motor patterns (fictive motor patterns)

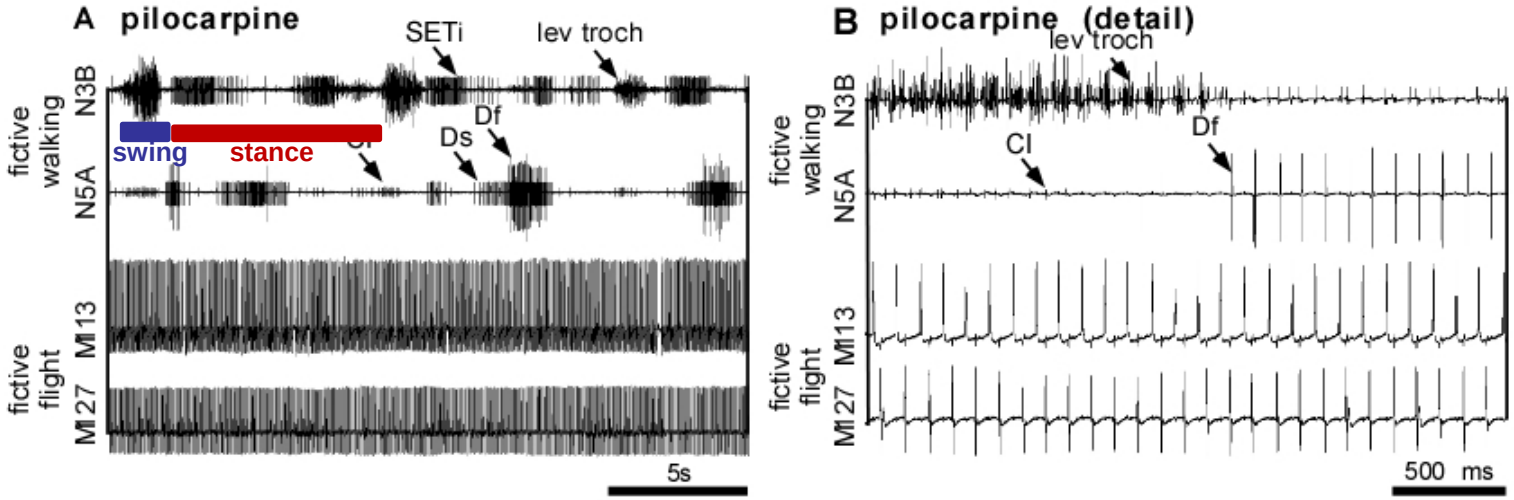


* pilocarpine: muscarinic acetylcholine receptor agonist

Fictive motor behaviours elicited in isolated thoracic ganglia (Central pattern generators)



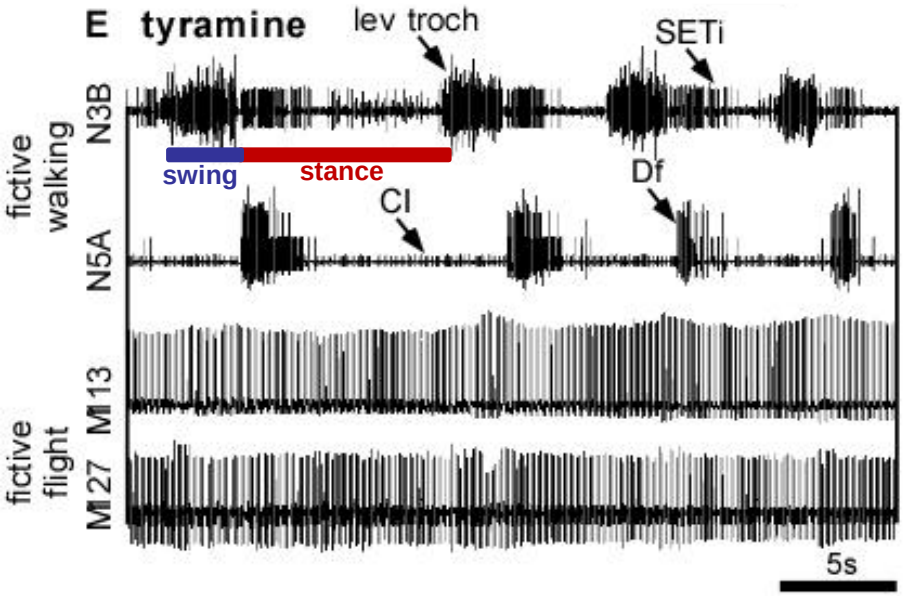
Fictive walking and fictive flight can be elicited by 10^{-3}M Pilocarpine* simultaneously

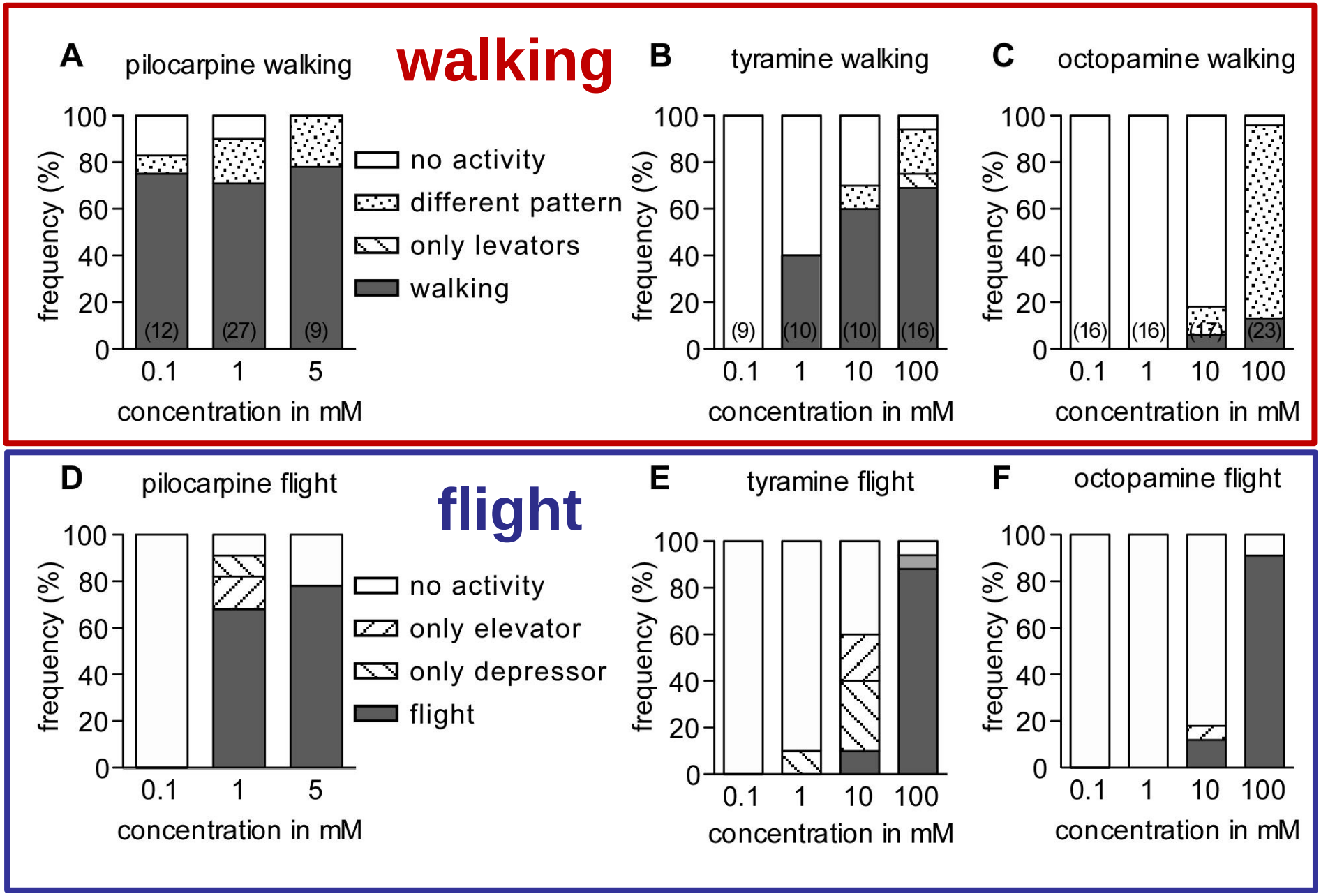


* pilocarpine: muscarinic acetylcholine receptor agonist

Tyramine can release a fictive flight and fictive walking pattern simultaneously.

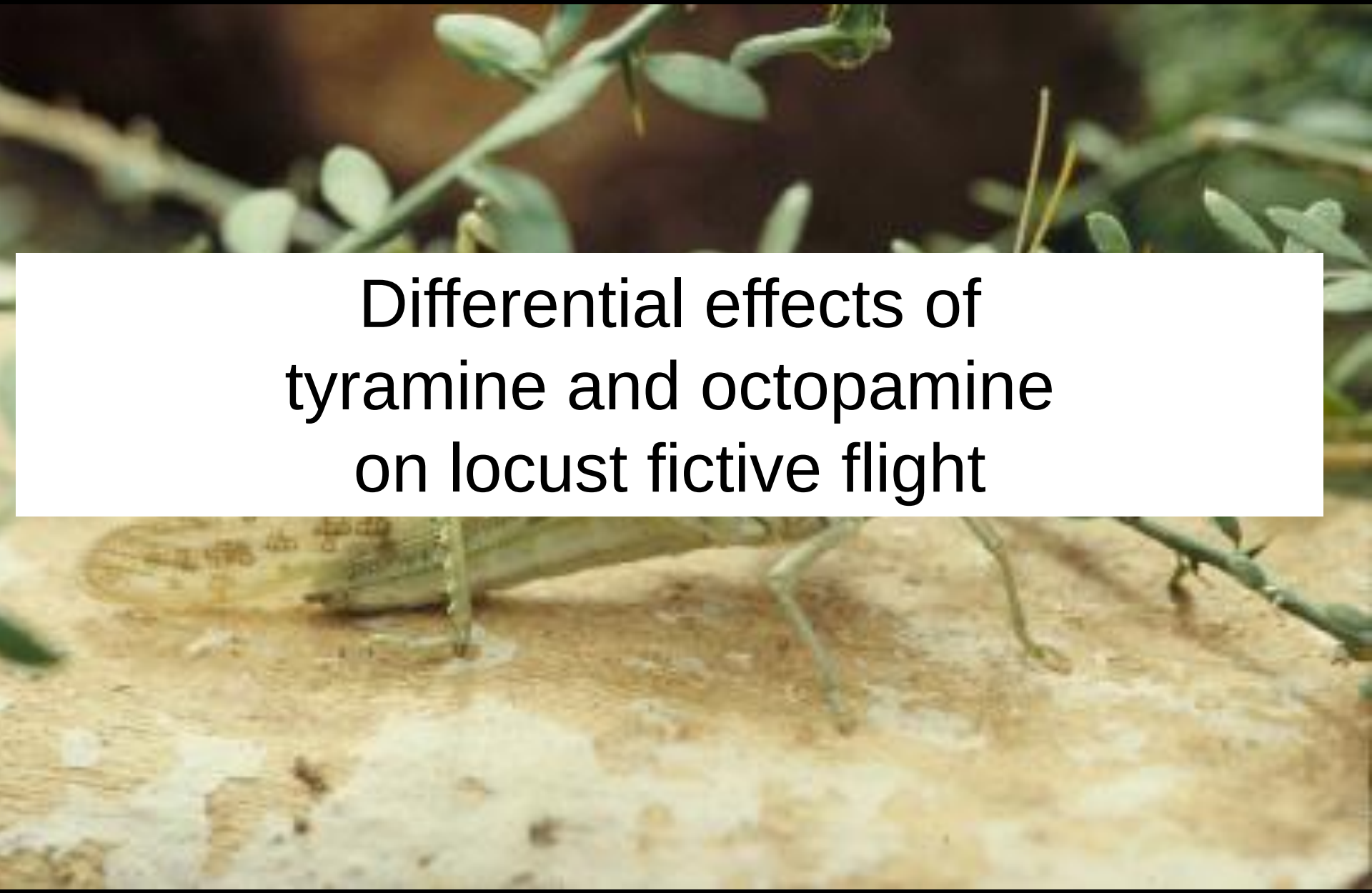
Octopamine only elicits a fictive flight pattern



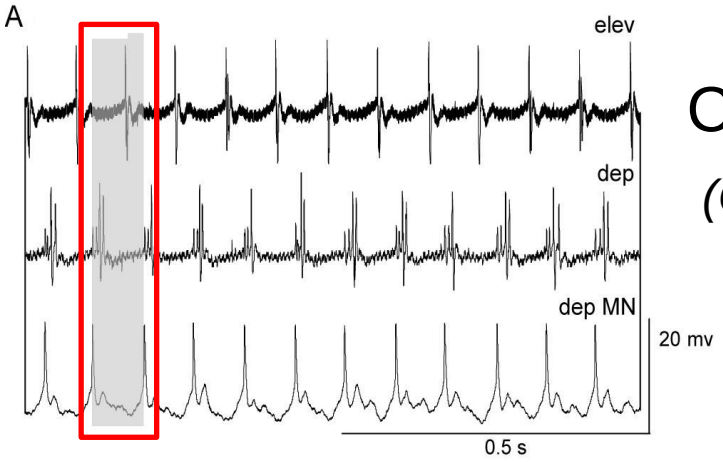

walking
A
pilocarpine walking
B
tyramine walking
C
octopamine walking
frequency (%)
100
80
60
40
20
0
(12)
(27)
(9)
(9)
(10)
(10)
(16)
(16)
(16)
(17)
(23)
0.1
1
5
10
100
concentration in mM
no activity
different pattern
only levators
walking
flight
D
pilocarpine flight
E
tyramine flight
F
octopamine flight
no activity
only elevator
only depressor
flight

* Tyramine has concentration-dependent effects on motor circuits:
  * low concentrations: walking is elicited
  * high concentrations: flight is elicited

* Octopamine (with very few exceptions, <10%) only elicits flight

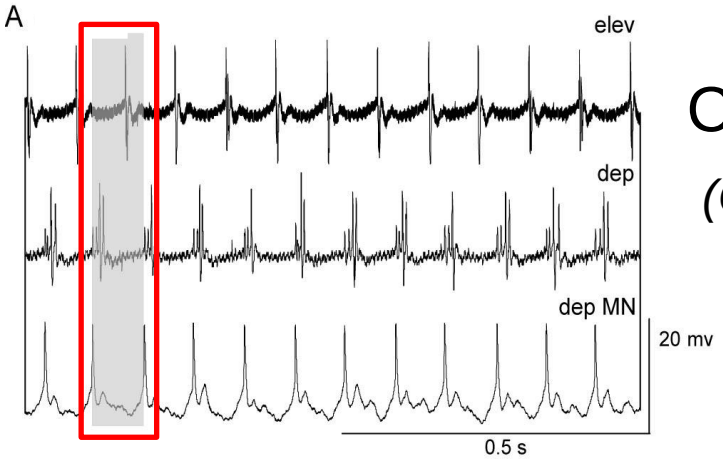


Differential effects of tyramine and octopamine on locust fictive flight



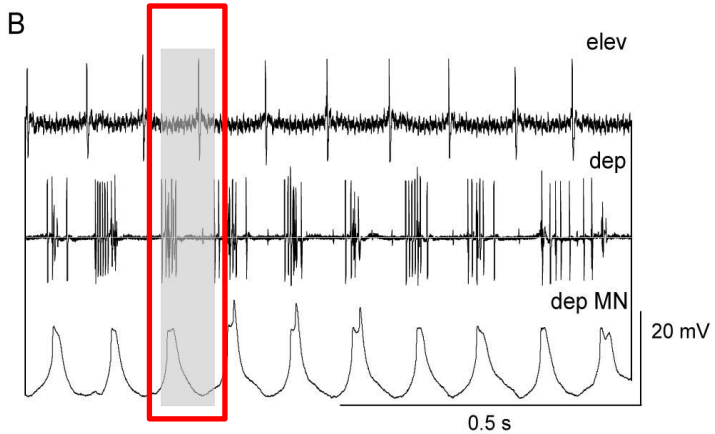
Chlordimeform 10^{-5} M

(OA-receptor agonist)

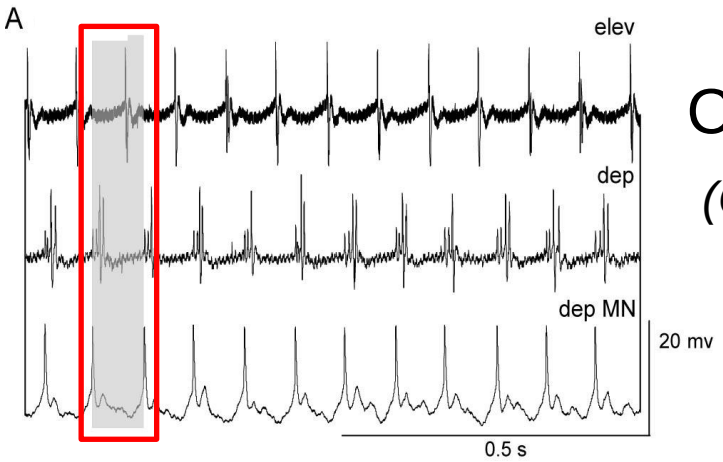


Chlordimeform 10^{-5} M

(OA-receptor agonist)

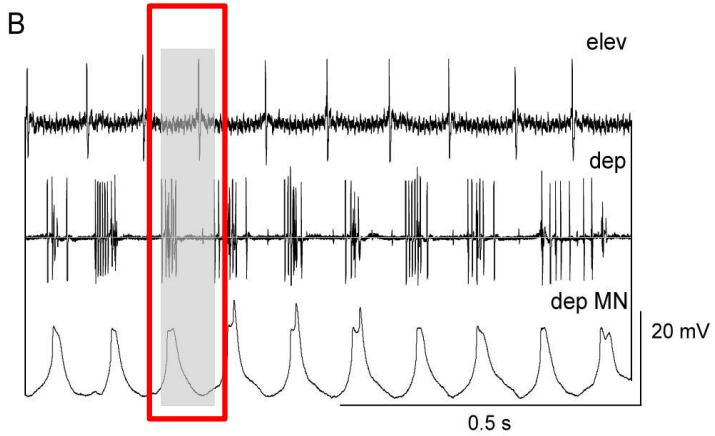


plus Tyramine 10^{-5} M



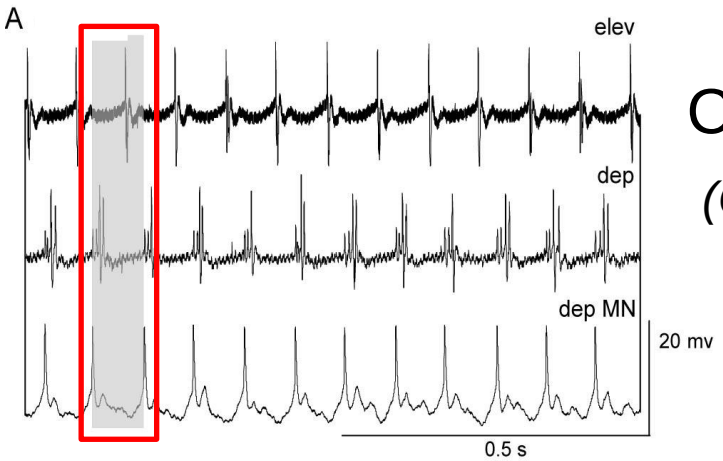
Chlordimeform 10^{-5} M

(OA-receptor agonist)



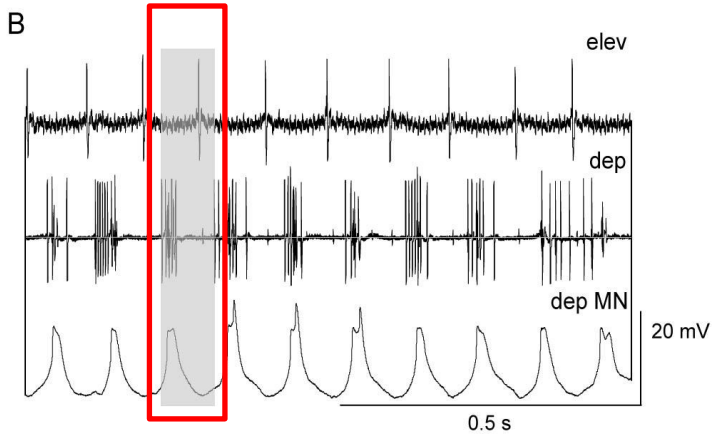
plus Tyramine 10^{-5} M

Recruitment of depressors,
Elevators unaffected



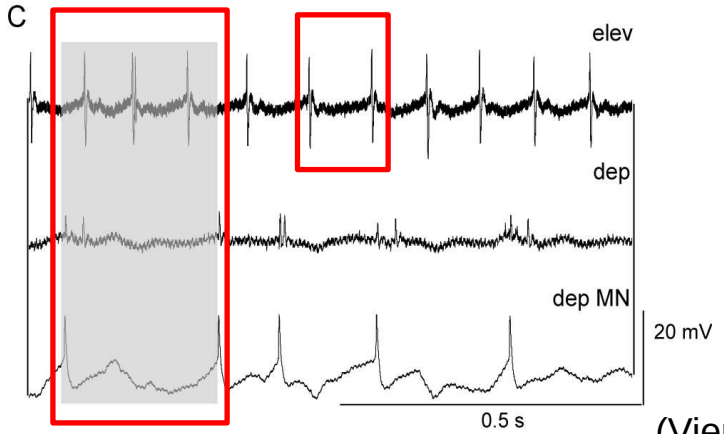
Chlordimeform 10^{-5} M

(OA-receptor agonist)



plus Tyramine 10^{-5} M

Recruitment of depressors,
Elevators unaffected



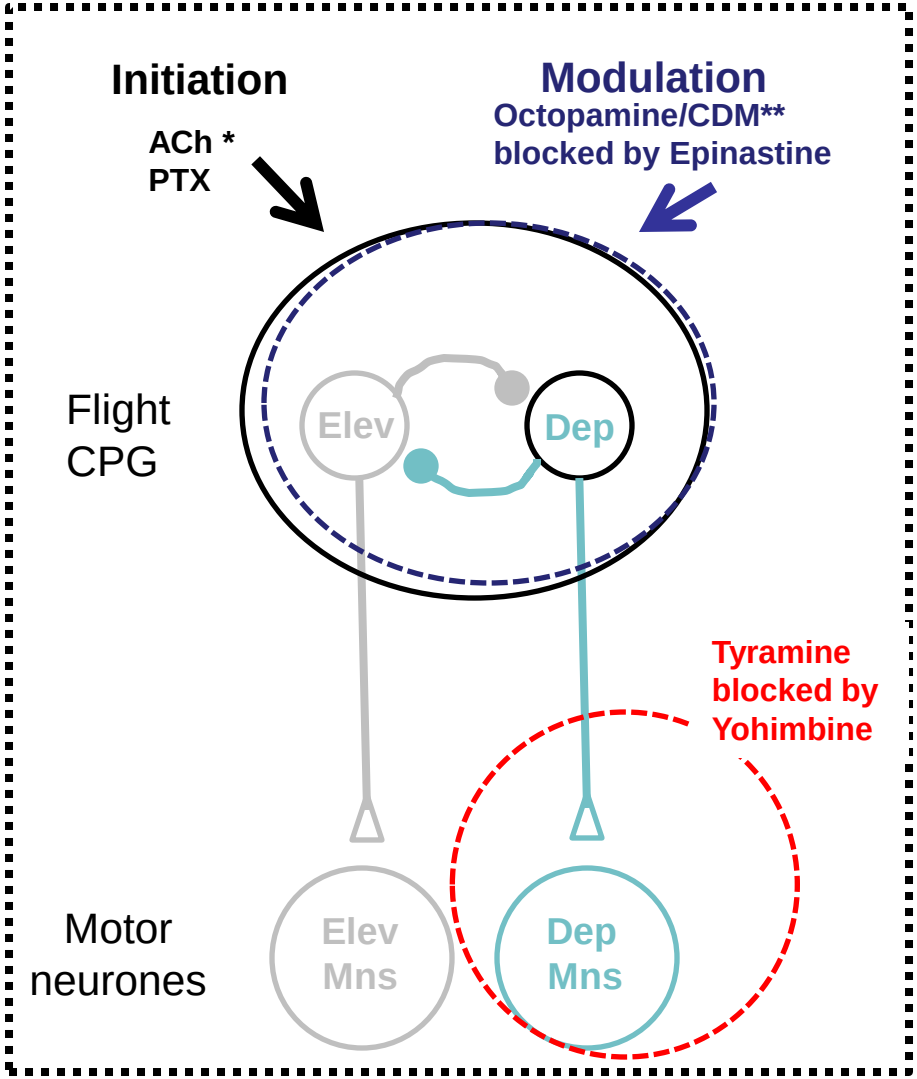
plus Yohimbine 10^{-5} M

(TA-receptor antagonist)

Inhibition of depressors,
Elevators unaffected

(Vierk et al. J Comp Physiol A, 195: 265-277, 2009)

Model



* Buhl, Schildberger, Stevenson, J Exp Biol 2008
** Vierk et al. JCP A 2009. and PhD-thesis R. Vierk

Summary

Central modulatory functions:

1) Amines such as tyramine, octopamine and dopamine are **modulators** of central pattern generators for locomotion, whereas acetylcholine is one of the **neurotransmitters** of the thoracic networks (*Buhl et al. JEB 2008).*

2) Fictive walking is induced by low concentrations of pilocarpine and tyramine (and dopamine, *Rillich unpublished*), and rarely by octopamine.

3) Fictive flight is induced by octopamine and by high concentrations of pilocarpine and tyramine (and dopamine, *but only for very short periods*).

Résumé:

Biogenic amines, such as octopamine „**orchestrate behavior**“ in the sense that they **coordinate all physiological and neuronal reponses**:

- react to particular **behavioral conditions** (**hunger, stress**, etc.)

- **coordinate** the **peripheral system** with respect to energy demands (*target tissue metabolism, efficacy of synaptic transmission, efficacy of proprioceptive sense organs, action on glands, all changed*)

- **coordinate** the **central networks** *(„arousal“, change probability for initiation and, thus, the appropriate behavior is generated*), and

- organize **meaningful behavior (**For example: *if animal is hungry, it has to forage, and search for food, and is also more aggressive*)

- Interestingly, the same amines are also used as the modulators involved in conditioning: **octopamine and dopamine in associative („Pavlovian“) conditioning, dopamine also in aversive conditioning.**
  (*see Waddell S, Curr Opin Neurobiol 201***3)**

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Forschungskommission Freie Universität Berlin

The neurons which release **tyramine** or **octopamine** (tyramine?) in insects (locust, *Schistocerca gregaria*)

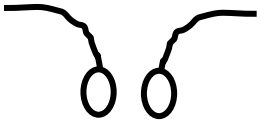
Neurons within the **CNS** (brain and ventral cord) *(„Interneurons“)*

- **Paired neurons in brain**

  *- 4 clusters of **OA** cells (1 in optic ganglia, at least 100), local, 1 cluster descending (~ 30)*

  *- 10 clusters of **TA** cells (3 in optical ganglia), local (~ 80)*

  (Kononenko et al., J Comp Neurol 512:433-452, 2009)



- **Paired neurons in ventral cord**

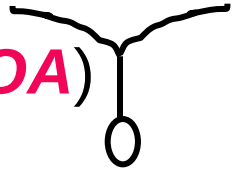
  *- 2 paired ventral lateral cells (**OA**), intersegmental (~ 10)*

  (Stevenson et al., J Comp Neurol 315, 382-397, 1992)

  *- 2 paired ventral cells in all fused ganglia (**TA**), intersegmental (~ 10)*

  (Kononenko et al., J Comp Neurol 512:433-452, 2009)

- **Unpaired neurons in suboesophageal ganglion** (*all **OA***)



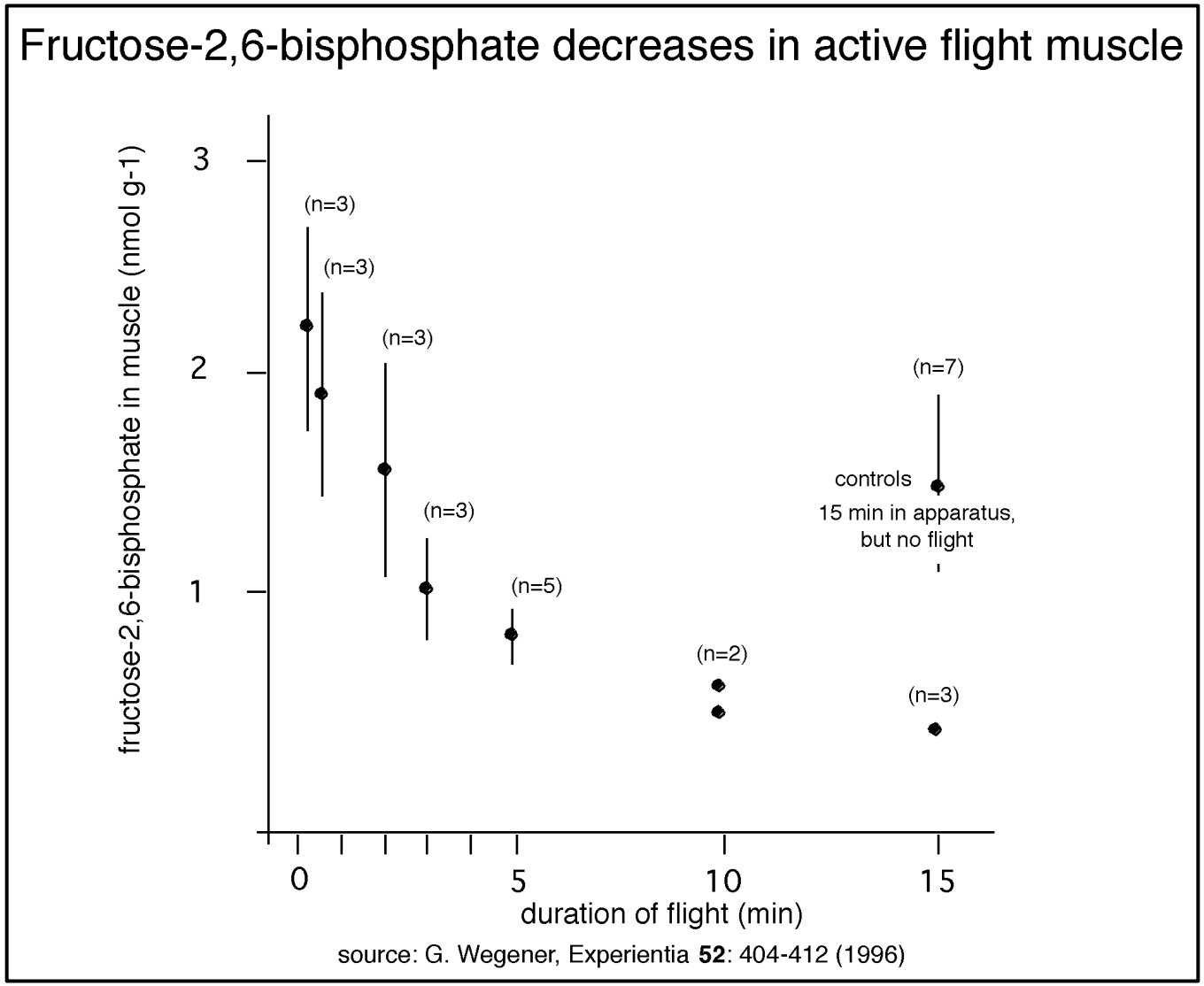
  - ascending to brain innervating all major neuropiles (9)

  (Bräunig, Phil Trans Roy Soc Lond B 332, 221-240,1991)

  - descending to ventral cord innervating thoracic and abdom. neuropiles (6)

  (Bräunig and Burrows, J Comp Neurol 478, 164-175, 2004)

However:



The F2,6BP – level in locust flight muscle after the onset of flight

- Discrepancy between the haemolymph data (OA↑) and the data from power flight muscle (OA↓)
- Muscles have to be specifically targeted (barrier to haemolymph?)

Fructose-2,6-bisphosphate decreases in active flight muscle

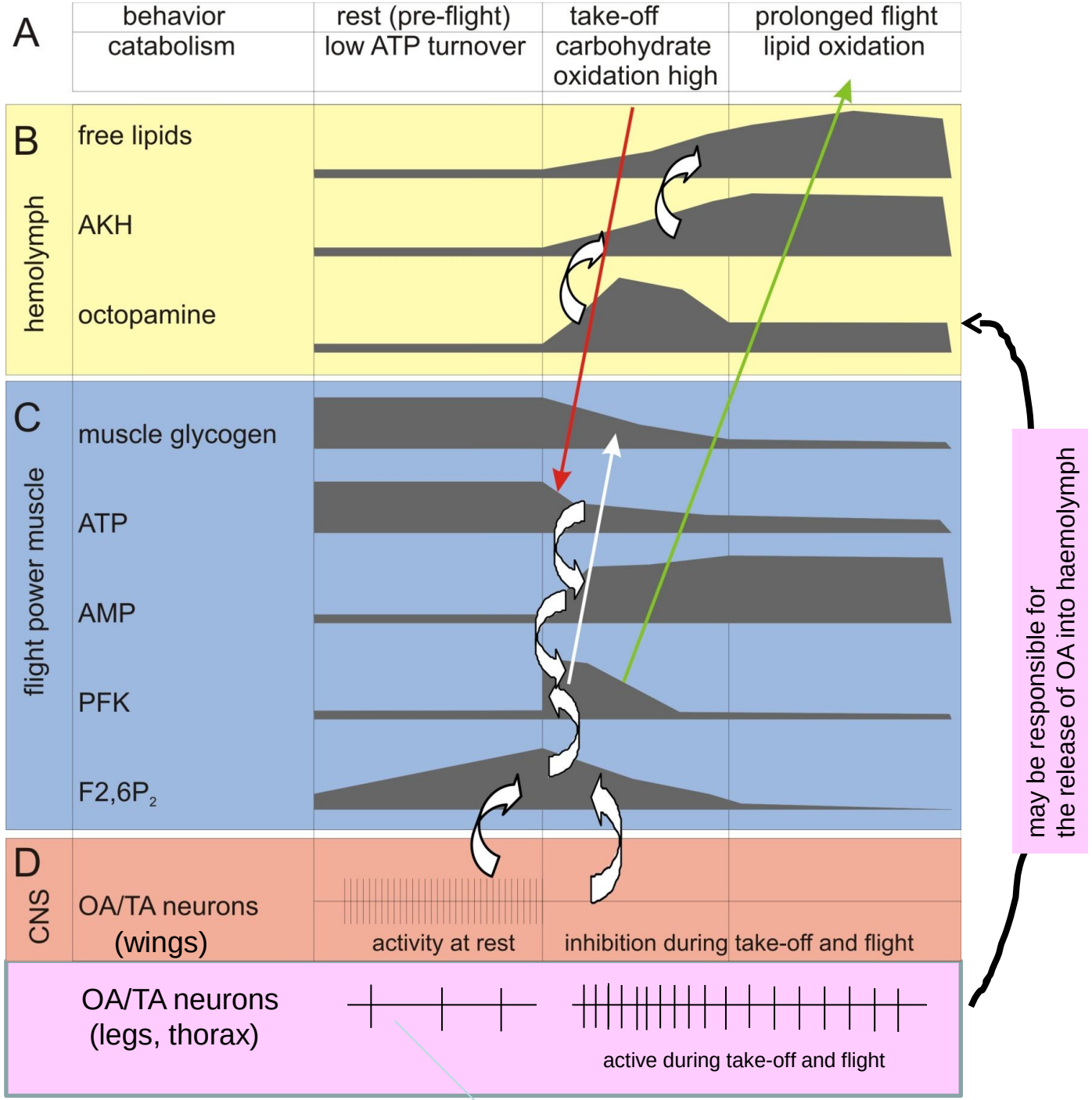
The discrepancy is explained by

* Wing muscles have their own supply by octopaminergic DUM neurons and these are switched off during flight

* Leg/Thorax muscles also have own supply by octopaminergic DUM neurons and these are activated during flight

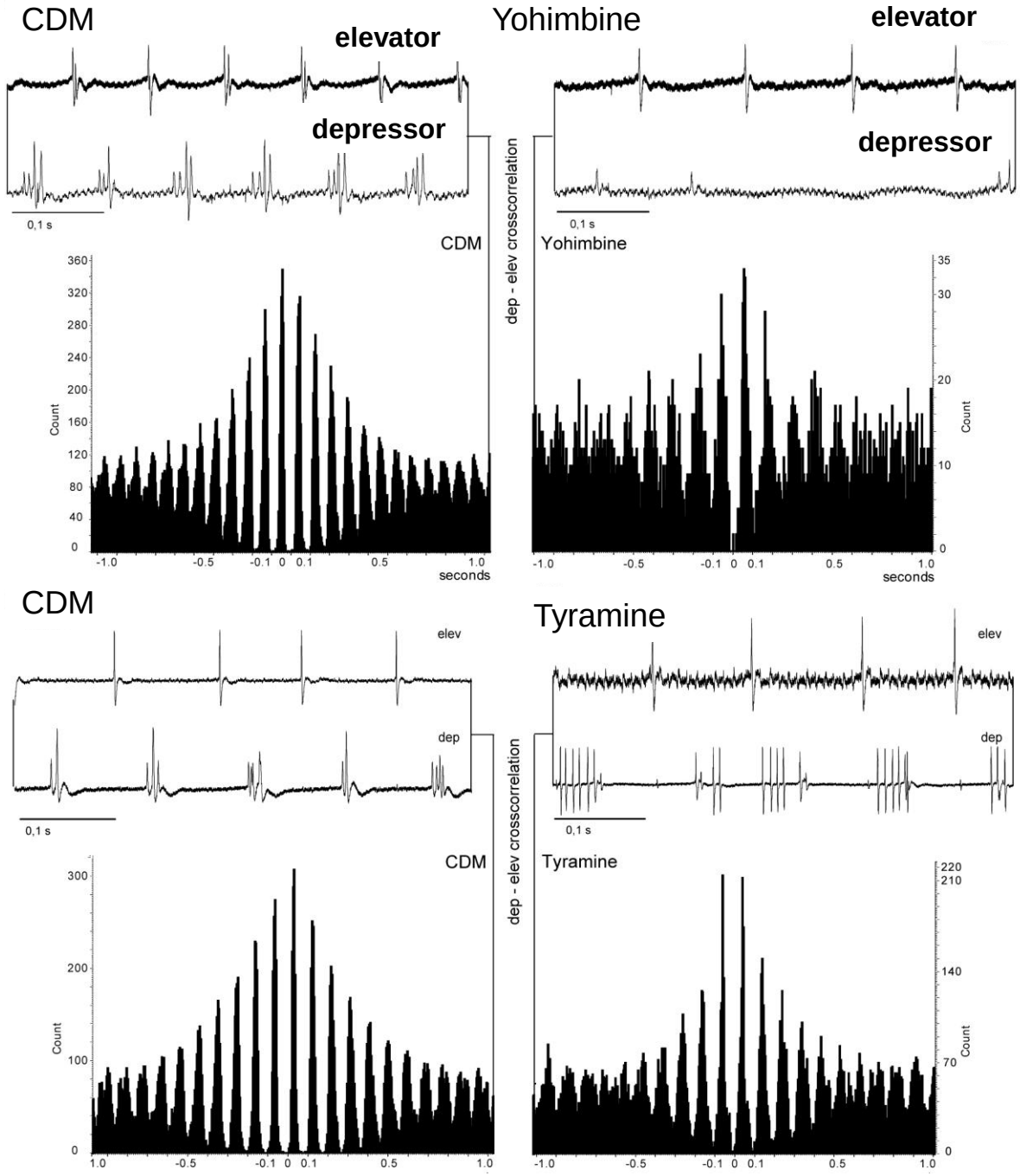
* The haemolymph octopamine could actually be released by the leg/thorax DUM or related DUM groups of neurons

data from Orchard, Ramirez et al.



Pflüger et al., 2004.
Acta Biol Hungarica

Pflüger and Duch, 2011,
Physiology



Precision of motor patterns
(auto-correlograms)